α-TOCOPHEROL IN MEAT AND MEAT PRODUCTS

INFLUENCE OF FEEDING ON α-TOCOPHEROL CONCENTRATION, QUALITY ATTRIBUTES AND STORAGE STABILITY

PH.D. THESIS

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α-TOCOPHEROL IN FLEISCH UND FLEISCHPRODUKTEN

EINFLUß DER FÜTTERUNG AUF α-TOCOPHEROL KONZENTRATION, QUALITÄTSPARAMETER UND LAGERSTABILITÄT

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Footprints

One night a man had a dream.
He dreamed he was walking along the beach with the LORD.
Across the sky flashed scenes from his life.
For each scene, he noticed two sets of footprints in the sand;
one belonging to him, and the other to the LORD.
When the last scene of his life flashed before him,
he looked back at the footprints in the sand.
He noticed that many times along the path of his life
there was only one set of footprints.
He also noticed that it happened at the very lowest and saddest times in his life.
This really bothered him and he questioned the LORD about it.
"LORD, you said that once I decided to follow you, you'd walk with me all the way.
But I've noticed that during the most troublesome times in my life,
there is only one set of footprints.
I don't understand why when I needed you most you would leave me."
The LORD replied, "My precious, precious child,
I love you and I would never leave you.
During your times of trial and suffering, when you see only one set of footprints,
it was then that I carried you."

Author unknown

Dedicated to all my foreign friends,
who have walked with me part of my way and helped me so much – they´ll never guess…

ABBREVIATIONS

BHT	butyl hydroxy toluene
CE	cholesterol esters
CHIS	Concentrate Headspace Injector System
DCM	dichloromethane
EtOH	ethanol
FAME	fatty acid methyl esters
FID	flame ionisation detector
GC	gaschromatography
GC-MS	gaschromatography - mass spectrometry
HDL	high density lipoproteins
HPLC	high performance liquid chromatography
ID	inner diameter
IS	internal standard
KOH	potassium hydroxide
LDL	low density lipoprotein
LW	life weight
MAK	maximum workplace concentration
ME	metabolisable energy
MeOH	methanol
MLD	*Musculus longissimus dorsi*
MST	*Musculus semitendinosus*
MUFA	monounsaturated fatty acids
n	number (of analysis)
n.d.	not detectable
p	probability (of mistake/error)
PL	phospholipids
PUFA	polyunsaturated fatty acids
RG	rapeseed glycerol
RL	rapeseed lecithin
RO	rapeseed oil
RP	reversed phase
SFA	saturated fatty acids
SL	soya lecithin
SP	straight phase
SPE	solid phase extraction
t	trans
TBARS	thiobarbituric acid reactive substances
TF	total fat
TG	triglycerides
UV	ultraviolet

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1 INTRODUCTION

Meat plays a very important role in the diet by contributing quality protein, essential minerals and trace elements, and a range of B vitamins. In addition to its nutritive value, meat has also attractive sensory properties. Despite these facts, meat consumption has come under close scrutiny in recent years. Dietary recommendations favouring the consumption of less saturated fats has led to an increase in demand for foods containing higher levels of unsaturated fatty acids. In addition, modern trends towards convenience foods have resulted in an elevated production of pre-cooked and restructured meat products. However, such foods are highly susceptible to lipid oxidation and off-flavour development and present the meat processor, distributor, and meat scientist with new challenges. Any improvements in meat quality are therefore of increased importance in view of stagnant or even decreasing trends in meat consumption in many western countries (BUCKLEY et al., 1995; ISSANCHOU, 1996).

When purchasing meat, the customer considers visual appearance as well as organoleptic criteria, e.g. smell, taste and tenderness, more than hygienic, technological or physiological factors. Post-slaughter changes due to oxidation have the most impact on meat quality. It is believed that oxidative changes are initiated at the membrane level, as its lipids are especially susceptible to oxidation by virtue of their high contents of highly unsaturated phospholipids (BUCKLEY et al., 1989). The self-catalytic free-radical reaction between these unsaturated fatty acids and molecular oxygen initiates the production of hydroperoxides, and propagates the production of reactive aldehydes. Moreover, reactions of generated radicals with proteins, pigments and additional lipids in the food system contribute to decreased nutritional quality, flavour deterioration and the presence of potentially toxic lipid oxidation products (KITTS, 1997).

It is well recognised that lipid oxidation in meat and meat products can be effectively controlled by antioxidants. Many studies have focused on the use of synthetic antioxidants. However, as consumers continue to become more health conscious, resistance to the use of synthetic antioxidants has increased. Instead, there is considerable interest in naturally occurring antioxidants, such as extracts from vegetables, fruits, grains, spices and herbs (BUCKLEY & MORRISSEY, 1992).
Tocopherols (or "Vitamin E") belong to this group, and they are especially important in slowing down *post mortem* oxidative changes due to their antioxidant properties (SCHAEFER et al., 1995). Several lines of evidence indicate that α-tocopherol is the most effective phenolic antioxidant for reducing lipid peroxidation in tissues. Especially membranes and low-density lipoproteins are protected (STAHL & SIES, 1996), when α-tocopherol is incorporated into the phospholipid bilayers of the membranes.

In order to achieve the incorporation into the tissue with a resulting maximum protection in meat, an oral application with the feed is superior to any kind of *post mortem* addition. Several studies have indicated that dietary supplementation with vitamin E increases the α-tocopherol concentration in blood plasma and tissues of humans and animals, mainly in dependence on

amount and duration of supplementation. But not only the level of the vitamin in meat of different origin was raised, also the level of lipid peroxides was lowered and the organoleptic properties were improved.

Beyond these facts considering meat quality, tocopherols are also widely discussed with respect to their influence on health aspects in human nutrition. They act directly or indirectly by reducing lipid oxidation and therefore decreasing possible toxicological effects of its products. This includes a variety of pathological processes, amongst others rheumatoid arthritis, atherosclerosis, mutagenesis and carcinogenesis (BUCKLEY & MORRISSEY, 1992) due to oxidative modification of low-density lipoproteins or cholesterol as well as free radicals. On the other hand no major negative effects have been reported for high levels of tocopherol uptake and it has become rather popular in recent years to consume tocopherols in any kind of preparation. As a consequence there is still a great interest in supplementing livestock feed with especially α-tocopherol in order to achieve a high quality meat product with a subsequent positive effect on human health.

1.1 Meat Quality

Historically, livestock nutrition has focused on issues of nutrient essentiality and feed efficiency. More recently, there has been an emphasis on dietary supplementation of food-producing animals to improve "quality" of the final product.
Several authors have tried to find a definition in order to describe meat quality in all its complexity. The following aspects appear to be relevant to most consumers and producers:

- hygiene (spoilage and pathogenic micro-organisms, contaminants and residues),
- food physiology (chemical composition, nutritive value),
- technology (fat content, content of connective tissue, water-binding properties, pH) and
- sensory (1. appearance: colour and its stability, shape and size, presentation;
  2. eating quality: tenderness, juiciness, smell and taste) and nowadays also the
- convenience (HOFMANN, 1987; GRAY et al., 1996; ISSANCHOU, 1996)

The three sensory properties by which consumers most readily judge meat quality are appearance, texture and flavour, but the most important one is the product's visual appearance as it strongly influences the consumer's purchase decision (LIU et al., 1995; GRAY et al., 1996).

The above listed properties are in general influenced by the animal's

- genetics (animal type, breed, sex),
- physiology (metabolism, state of health, use of drugs),
- anatomy (age, weight, connective tissue, fat distribution),
- production (feeding, growing conditions, stress, pre-slaughter treatment),
- post-slaughter treatment (temperature, pH, storage, maturity, tenderiser, freezing, hygiene),
- preparation (chopping, grinding, cooking, sterilisation, spices, additives, curing, etc.) (HOFMANN, 1987; ENDER, 1990).

Post-slaughter changes due to oxidation have the most impact on meat colour and therefore, on the first visual impression. The colour changes from cherry-red, caused by oxymyoglobin, to a brownish colour, due to metmyoglobin (LIU et al., 1995). The oxidation of fat is the cause for changes in smell, taste, texture and nutritional value of meat, which consequently determines meat quality to a wide extent (AUGUSTINI, 1998). From unsaturated fatty acids hydroperoxides are arising by auto-oxidation reactions. These hydroperoxides are precursors to a complex mixture of volatile substances, which on the one hand determine the aroma profile of meat, on the other hand they can cause sensory defects, called "off-flavour" (GROSCH, 1989). For this reason the phospholipids should be considered for the evaluation of sensory meat quality (BLUNK et al., 1992). Another consequence of lipid oxidation is the reaction of peroxidised lipids with amino acids and proteins, which causes a decreasing quality of texture (cross-linking, polymerisation, denaturation and inhibition of enzymes), a loss of the nutritional value and a significant generation of toxins (KANNER, 1994).

1.2 Fatty Acids and Lipids in Meat

The lipid portion of muscle foods is associated with numerous processing or product variables. These include flavour, colour, stability, texture juiciness, protein stability, frozen storage shelf-life, emulsion characteristics and caloric content (ALLEN & FOEGEDING, 1981).

Meat lipids are present as phospholipids of the membranes of the meat tissue (structural lipids) and in the storage fat (adipose tissue) associated with the meat. Some storage fat is located within the muscle tissue (marbling). The phospholipid and cholesterol portions of muscle lipids are essential because of their role in the structure and function of the muscle cell and its organelles. The structural lipids represent only a small proportion of total lipid but contribute to a large proportion of meat polyunsaturated fatty acids (PUFA). The storage fats are generally present in larger amounts but have a higher proportion of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). These neutral lipids are not essential, but they do provide fatty acids for energy metabolism in living muscle and contribute to the characteristics of the meat (ALLEN & FOEGEDING, 1981; GURR, 1997).
Fatty acid patterns are different for adipose tissue and intramuscular fat because of their special physiological functions, so the amounts of MUFA and PUFA are divergent (FLACHOWSKY et al., 1994). Fatty acids in blood also differ, as this is a transport rather than a storage or structure organ. Particularly interesting are the fatty acid compositions of the phospholipid fractions. The phospholipids are mainly localised in cell membranes and due to their high content of PUFA they are extremely sensitive to oxidation processes and therefore more important for aroma than neutral fats (LARICK et al., 1989; LÜBBE, 1990).

In all of the non-ruminant species dietary fatty acids are very important in determining tissue fatty acid composition, whereas among ruminants it now is also possible to produce polyunsaturated meat products by feeding "protected" (encapsulated) oilseed supplements (ALLEN & FOEGEDING, 1981). A determination of the fatty acid pattern of meat therefore might reflect feeding effects, as well as the blood fatty acid composition can give information about the

actual situation and uptake from feed. This can be an indication for lipid oxidation and the resulting quality of the meat that is to be expected.

A main characteristic of lipids is that they are precursors for aroma compounds. These are especially arising through oxidation reactions and determine smell and taste (GROSCH, 1989). According to BLUNK et al. (1992) phospholipids are the most important precursors for volatiles due to their high content of PUFA. Upon exposure to the atmosphere, phospholipids extracted from pork and beef muscles develop rancid off-flavours much faster than neutral fats. This high susceptibility of phospholipids to oxidation is attributed to their high concentration of PUFA (LARICK et al., 1989). According to GURR (1997) meats with increased content of PUFA have therefore not become established in the market place mainly because of short shelf-life of products due to oxidative rancidity.

1.2.1 Lipid Oxidation

Any disruption of the integrity of muscle membranes by mechanical deboning, grinding, restructuring or cooking alters the cellular compartmentalisation. This facilitates the interactions of pro-oxidants with unsaturated fatty acids resulting in the generation of free radicals and the propagation of oxidative reactions. Lipid oxidation is one of the primary mechanisms of quality deterioration in foods and especially in meat products. The changes in quality are manifested by adverse changes in flavour, colour, texture and nutritive value, and in the possible production of toxic compounds (GRAY et al., 1996)

Free radicals are an integral part of the food that we eat or they may be produced through the oxidative processes within the body. Once started, they can damage both the structure and function of a cell membrane in a free radical chain reaction leading up to degenerative diseases and conditions (AHMAD, 1996).
Three main stages can be identified within the mechanism: initiation, propagation and termination. In the presence of initiators, unsaturated lipids (LH) form carbon-centred alkyl radicals (L*, reaction 1) and peroxyl radicals (LOO*, reaction 2), which propagate in the presence of oxygen.

$$LH + O_2 \rightarrow L^* + HO_2^* \qquad (1)$$
$$L^* + O_2 \rightarrow LOO^* \qquad (2)$$

In the presence of light, unsaturated fats can also form hydroperoxides (LOOH) by reacting with the short-lived but highly reactive, high energy form of singlet oxygen $^1O_2$. In food systems these molecules arise e.g. from reactions between low energy, ground state oxygen (triplet oxygen $^3O_2$) and pigments such as chlorophyll, riboflavin or haem, in the presence of light (FRANKEL, 1991; AHMAD, 1996). Or a variety of radicals arises by the reduction of oxygen, such as superoxide anion radical (O_2^{-}*), perhydroxyl radical (HO_2*), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO*) all of which may participate directly or indirectly in oxidative processes in meat (KANNER, 1994).

The singlet oxygen or one of the radicals then adds to the olefinic carbon of an unsaturated fatty acid (in a membrane or lipoprotein). This gives rise to a hydroperoxide (LOOH, reaction 3 or 4) and terminates the chain reaction (the same effect is achieved when two radicals join and form a non-radical product). But in the presence of traces of transition metals, these primary oxidation products are readily decomposed to form alkoxyl radical intermediates (LO* and LOO*, reactions 5 and 6), which can effectively propagate the free radical chain (FRANKEL, 1991).

$$O_2 + LH \rightarrow LOOH \quad (3)$$
$$LOO^* + LH \rightarrow LOOH + L^* \quad (4)$$
$$LOOH + M^n \rightarrow LO^* + OH^- + M^{n+1} \quad (5)$$
$$LOOH + M^{n+1} \rightarrow LOO^* + H^+ + M^n \quad (6)$$

Hydroperoxides are essentially odourless. They can as well accumulate in the oxidising fat, but as the chain reaction proceeds, the build-up of their breakdown products becomes increasingly important. In the autoxidation reaction these first hydroperoxides spontaneously decompose to hydroperoxy and alkoxy radicals and a variety of volatile and non-volatile secondary products. The alkoxy radicals give rise to a wide range of carbonyl compounds (aldehydes, ketones and alcohols), hydrocarbons and furans that contribute to flavour deterioration as well as continuing the supply of free radicals to maintain the chain reaction (KANNER, 1994; AHMAD, 1996; GRAY et al., 1996; KITTS, 1997). Aldehydes arising from the cleavage of the carbon chain on either side of the alkoxy radical are the source of rancid fat's characteristic odour, which in general is called "off-flavour" (AHMAD, 1996). In food systems, the rate of autoxidation depends on temperature, pH, the degree of unsaturation of the fatty acids, as well as on the availability of oxygen and transition metal ions (KITTS, 1997).

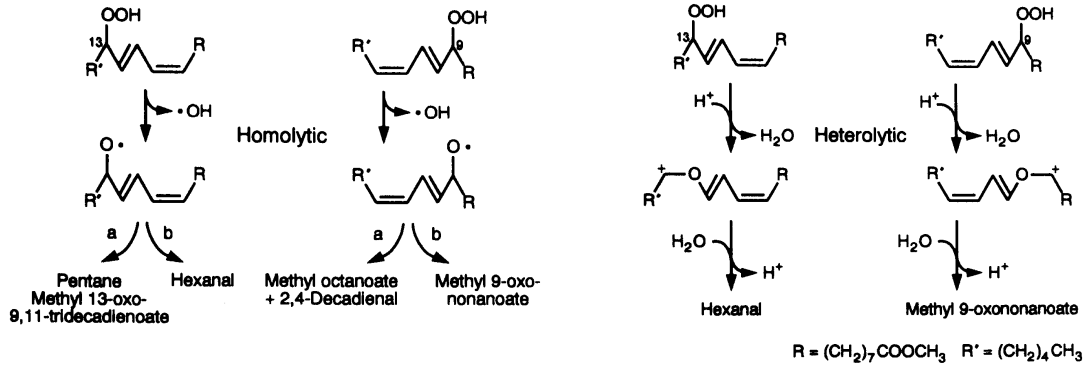

Figure 1: Homolytic and heterolytic decomposition of hydroperoxides of methyl linoleate (FRANKEL, 1991)

The main cleavage mechanisms recognised for 13-linoleate hydroperoxide produce hexanal and pentane, and from the 9-linoleate hydroperoxide, methyl 9-oxononanoate, 2,4-decadienal and methyl octanoate (see fig. 1). Linolenate hydroperoxides mainly decompose to propanol, methyl 9-oxononanoate, methyl octanoate and 2,4,7-decatrienal. The use of hexanal to determine the course of lipid oxidation is frequently used and hexanal was proposed as an indicator of oxidative stability and flavour acceptability (KANNER, 1994).

The susceptibility of meat products to lipid oxidation has challenged the meat technologist to come up with techniques to extend the shelf-life and maintain the nutritional quality of these lipid-containing foods. Much attention has focused on packaging and both vacuum packaging and modified atmosphere packaging have proved very effective. Many studies have indicated that lipid oxidation can be effectively controlled or, at least, minimised by the use of antioxidants (GRAY et al., 1996). Food nutrients such as ascorbic acid, tocopherols, and plant's phenolic compounds (e.g. carotenoids, flavonoids, rosmarinic acid, etc.) have antioxidant abilities. At low concentrations (HALLIWELL et al., 1995) they interfere with the normal oxidation process and delay it by reacting with either chain-carrying peroxyl radicals or the alkyl radical intermediates.

$$LOO^* + AH \rightarrow LOOH + A^* \quad (7)$$

$$L^* + Q \rightarrow LQ^* \quad (8)$$

Primary antioxidants (AH) include hindered phenols such as butylated hydroxyanisole, butylated hydroxy toluene (BHT) and natural tocopherols. To be effective in protecting the structural integrity of the membranes as well as the optimum cell function, they must compete with the unsaturated lipid substrate (reaction 3) and act as electron/hydrogen donors to terminate the free radical chain (reaction 7; AHMAD, 1996). A second class of antioxidants (Q, reaction 8) includes quinones, which must compete with O_2 in the fast reaction (2). These compounds may therefore only be active in biological systems where the oxygen pressure is relatively low (FRANKEL, 1991). As the resulting radicals A* and LQ* are stabilised by resonance their reactivity is declined and the chain reaction will be slowed down.

1.2.2 Health Aspects of Fat and Fatty Acids

It is generally considered that excess nutrients are hazardous for human health. Especially excess intake of beef fat induces hyper-lipidemia and cholesteraemia, which result in coronary heart disease and cancer (TAJIMA et al., 1995).

There has been a comprehensive public debate about the relationship between the fatty acid composition of human diets and the incidence of coronary heart disease. The risks of coronary heart complications seem to be reduced by lowering the dietary cholesterol and fat caloric intake (MALMFORS et al., 1978). High levels of plasma cholesterol are influenced by the composition of dietary fat. Therefore it has been recommended to substitute saturated animal fats with unsaturated plant oils in order to increase the intake of PUFA and decrease the uptake of SFA (MORGAN et al., 1992; TAJIMA et al., 1995).

A major justification for decreasing dietary fat has been the anticipated reductions in cancer of the breast, colon and prostate. In most human case-control studies a weak positive association has been observed between fat intake and breast cancer. Positive associations between animal (but not vegetable) fat consumption and colon cancer incidence have also been seen in many studies. Large differences in cancer rates are probably due to multiple factors that include reproductive pattern, physical activity, adiposity, alcohol intake, and use of exogenous estrogen.

A conclusions can be drawn that intake of partially hydrogenated vegetable fats and saturated fats, particularly those from dairy sources, should be minimised (WILLETT, 1994).

PUFA are supposed to have a favourable influence on the incidence of cardiovascular disease in humans. The frequently occurring linoleic and linolenic acid are essential. Few of the essential PUFA are either used for cell-mediator synthesis or play a structural role in membranes (WARNANTS et al., 1995). The optimal amount of polyunsaturated fat intake remains uncertain. When the effects of polyunsaturated fat on blood lipids are the primary consideration, it appears that intakes should be maximised. However, concerns have arisen because high intakes of ω6–PUFA might promote tumours and coronary thrombosis by competing with ω3–PUFA (WILLETT, 1994).

Definitive data are not available on the optimal intake of PUFA and MUFA, but metabolic data and experiences suggest that consumption of a substantial proportion of energy as monounsaturated fat would not be harmful and might even be beneficial (WILLETT, 1994). Ideally, the amount of fat consumed should be no more than 30 % of the daily energy intake and should be equally divided between SFA, MUFA and PUFA (WARNANTS et al., 1995). It has been suggested that an increased daily intake of long chain ω3–PUFA to 0.5-1.0 g per day would reduce risk of cardiovascular death. Increasing the intake of the ω3–PUFA precursor, α-linolenic acid, will lead to increased ω3-PUFA in plasma and show anti-aggregatory effects on platelets. The slow desaturation and elongation rates for C_{18}-fatty acids in humans and decreased desaturase activity with ageing make direct dietary supplementation of long chain ω3-PUFA more attractive (O´KEEFE et al., 1995).

A negative health aspect of markedly increasing PUFA, in particular ω3-PUFA, is possible. Potential human health risks are associated with increased consumption of ω3–PUFA due to increased consumption of lipid oxidation products, increased in vivo production of lipid oxidation products and depletion of tissue levels of vitamin E. Therefore the storage stability of shelf-life and safety of ω3–PUFA enriched meats should be considered when evaluating their nutritional and functional values and consumer acceptance (AJUYAH et al., 1993a).

Understanding the interrelations between dietary fats, blood lipids, and risk of coronary heart disease has been further complicated by evidence that antioxidants may protect against atherosclerosis. Antioxidants such as vitamin E have been shown to block the oxidative modification of low-density lipoprotein (LDL), an important step in atherogenesis. The maximum reduction in risk was associated with vitamin E intakes of >100 mg per day - well above the levels achievable by diet alone (WILLETT, 1994).

1.3 Antioxidants

Antioxidants are used to prolong shelf-life and maintain the nutritional quality of lipid containing foods as well as to modulate the consequences of oxidative damage in the human body. Often the term "antioxidant" is implicitly restricted to chain breaking inhibitors of lipid peroxidation, such as α-tocopherol. Hence a broader definition is "any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate" (HALLIWELL et al., 1995).
They may be classified as follows (AHMAD, 1996):

- Primary antioxidants are mainly phenolic and terminate the free radical chain by acting as an electron donor. The free antioxidant radical is stabilised by resonance rendering it insufficiently reactive to continue in the chain reaction. Substances such as natural and synthetic tocopherols, alkyl gallates, butylated hydroxyanisole or butylated hydroxy toluene belong to this group.
- Oxygen scavengers such as ascorbic acid (vitamin C) and ascorbyl palmitate react with oxygen and thus remove it from a closed system.
- Secondary antioxidants function by decomposing the lipid hydroperoxides into stable end products. Dilauryl thiopropionate and thiopropionic acid are examples for this group.
- Enzymic antioxidants function either by removing dissolved or headspace oxygen (e.g. glucose oxidase) or by removing highly oxidative species from food systems e.g. with superoxidase dismutase and catalase.
- Chelating agents stop metallic ions such as iron and copper from promoting lipid oxidation by chelating them. Examples are citric acid, amino acids and ethylenediamine tetra acetic acid.

A wide range of compounds has been suggested to act as antioxidants *in vivo* and in foods - from butylated hydroxy toluene, butylated hydroxyanisole, β-carotene and metallothionein to histidine-containing dipeptides (carnosine, anserine), taurine, oestrogens, creatinine, polyamines and melatonin. Antioxidant compounds derived from plants, especially phenols such as flavonoids, e.g. quercetin or catechins, carnosol, thymol, gallic acid derivatives, tannins, vanillin, eugenol and rosmarinic acids among others, are of considerable interest from the viewpoint of dietary antioxidant supplementation and food preservation.

It is perfectly possible for an antioxidant to protect in one system but fail to protect, or even sometimes to cause damage, in others. It is also possible that the antioxidant activity of some lipid-soluble chain-breaking antioxidants might appear to be greater in systems containing ascorbic acid (HALLIWELL et al., 1995). In its natural forms, ascorbic acid (vitamin C) functions both as an oxygen scavenger and as a reducing agent. The latter is important for the regeneration of the metabolically active form of tocopherol from the semi-stable tocopheroxyl radical. In addition, the metal-sequestering activity of ascorbic acid, which forms complexes that are less reactive with oxygen, provides antioxidant activity (KITTS, 1997).
These synergistic interactions of vitamin C, vitamin E, carotenoids, flavonoids, selenium and other antioxidants present in (or added to) foods are very important, not only in preventing rancidity but also in maintaining the nutritional quality of the food by keeping high levels of the

antioxidant vitamins (HALLIWELL et al., 1995). In several studies it could be shown that supplementation by a combination of antioxidants provides stronger protection against oxidative damage to tissues than supplementation by an individual antioxidant.

1.3.1 Tocopherols

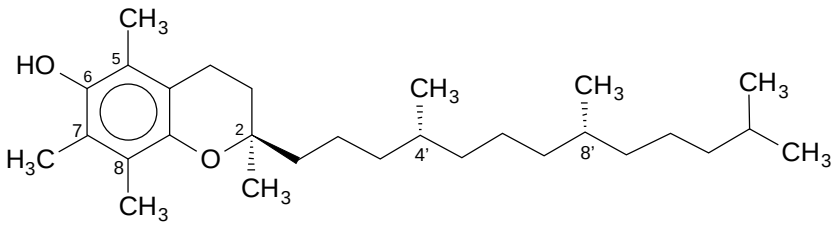

Figure 2: L-α-Tocopherol (R,R,R)

"Tocopherol" or "Vitamin E" refers to a group of eight naturally occurring tocochromanols: alpha-, beta-, gamma- and delta-tocopherol and the corresponding tocotrienols. Tocopherols are a group of derivatives of 6-chromanol with a C_{16}-phytyl side chain, tocotrienols possess the corresponding three-fold unsaturated side chain. The homologues α-, β-, γ- and δ-tocopherol and -tocotrienol, respectively, differ in their degree and position of methyl substitution (C_5, C_7 or C_8 of the chromanol ring). Natural α-tocopherol consists of a single stereo isomer, 2R,4´R,8´R-α-tocopherol (see fig. 2). Owing to 3 asymmetric centres in the side chain, totally synthetic tocopherols are composed of equal parts of eight stereo isomers, leading to a group of already 32 tocopherols, which have not yet been studied individually in detail.

Unfortunately, in various publications the terms for the different compounds are sometimes used in different ways or their use is neither precise nor systematic or consequent, and the exact composition of tocopherols (especially of the stereo isomers) is often not specified or not known. This might lead to misunderstandings, differing interpretation or simply different results.
In this paper, the term "vitamin E" will subsequently be used either as generic term when referring to all tocochromanols or when there was no additional information given in the publications cited, whereas "tocopherol" is reserved as synonym for the eight stereo isomers of one of the tocopherol homologues, e.g. α-tocopherol.

All the eight tocochromanols mentioned have been isolated from vegetable oils and other plant materials, which are the major contributors of dietary vitamin E. Concentrations in animal fats are generally much lower than in vegetable oils and fats, even after refining and hydrogenation of the latter (PARKER, 1989). They occur as viscous, yellow, fat soluble oils and are resistant to heat and alkaline in absence of oxidising influences, which makes them useful as stable nutrients. Because humans and animals are unable to synthesise vitamin E they have to rely on obtaining it from other sources.

The most important commercial compounds are alpha-, gamma- and mixed tocopherols. The former two are commonly synthesised and the latter is a by-product of vegetable oil processing (JOHNSON, 1995). Enrichment of foods and feeds is usually performed with all-*rac*-α-tocopheryl acetate or other esters because these are very stable (SCHÜEP & RETTENMAIER, 1994; LIU et al., 1995). 60-70 % of the annual production are used for supplementation of feeds, either on a basic level to prevent from deficiency diseases or in higher amounts to increase the value and stabilise the tissues (ROSENBAUER et al., 1996). In contrast to technical antioxidants, the application is not restricted by law, but due to high costs it is not commonly used (KREUZER, 1993).

Requirements and utilisation of vitamin E varies according to individuals. The ability to absorb vitamin E is linked to the ability to absorb triglycerides. Dietary vitamin E is taken up via the lymphatic pathway. Orally administered α-tocopherol is first secreted from the intestine in chylomicrons, then is secreted from the liver in very-low-density lipoproteins (VLDL) and appears in the plasma simultaneously in low- and high-density lipoproteins (LDL and HDL, respectively). Due to the presence of a transfer protein in liver a selective release of natural R,R,R-α-tocopherol into the systemic circulation takes place, whereas the other forms are preferentially excreted, therefore this α-tocopherol isomer is the predominant component in human and animal tissue and blood. The fractional absorption in humans has been estimated to be about 70 % (KAYDEN & TRABER, 1993; STAHL & SIES, 1996). The tocopheryl esters are normally very non-reactive under the most common conditions of food processing and do not function as antioxidants until they are de-esterified in the gastrointestinal tract. HIDIROGLOU et al. (1988) found out in their beef experiments though, that concerning its uptake, RRR-α-tocopherol is yielding the greatest response, followed by its acetate ester and then the racemic products. On the other hand BURTON et al. (1988) determined for rats the net uptake of α-tocopherol from the free phenol form to be only half that from the acetate, whereas in humans both forms were equal. The position of methyl substitution is the reason for the different degree of biologic and antioxidant activities of the tocopherols. The order of antioxidant efficiency can vary depending on test conditions (JOHNSON, 1995). Considering the biological activity, RRR-α-tocopherol (see fig. 2) is the most active substance compared to its stereo isomers and other tocopherols (AHMAD, 1996).

Tocopherols stand as the first line of defence against lipid peroxidation because of its radical quenching ability and prevents the cell membrane from free radical attack (AHMAD, 1996). The antioxidative effect of tocopherols is due to its hydroxyl group and its incorporation into the biological cell membranes. When incorporated into the lipid bilayers of the membrane α-tocopherol is located next to the sensitive phospholipids: the polar chromanol ring is placed at the membrane surface between the polar regions of the phospholipids whereas the phytyl side chain interacts with the fatty acids in the inner part of the membrane and serves to anchor and position the vitamin perpendicular to the plane of the bilayer (PARKER, 1989).
The hydroxyl group is acting as a hydrogen donor to stabilise free radicals (see also 1.2.1, reaction 7), which makes itself to become a tocopheroxyl radical, which is relatively unreactive with molecular oxygen and organic compounds, such as PUFA, because it is stabilised by resonance. This way it is acting as a free radical scavenger, the chain transfer (propagation) reaction is retarded and therefore especially the unsaturated fatty acids in the phospholipids are

protected from lipid peroxidation which would lead to unwanted lipid oxidation products. The efficient antioxidative activity of α-tocopherol despite its low concentration in biological membranes (relative to unsaturated fatty acids) is due to the fact, that it is immediately regenerated from the radical, most likely by ascorbic acid. In this way, α-tocopherol is not metabolised, but instead is maintained in the body in the unoxidised state (PARKER, 1989; KAYDEN & TRABER, 1993).

1.3.2 Health Aspects of Tocopherols

The role of vitamin E in human health concerning requirements and utilisation varies according to individuals. Deficiency symptoms are long term and do not manifest readily and are linked to degenerative diseases such as cancer, atherosclerosis and other forms of heart diseases.

AHMAD (1996) has reviewed the different modes of operation of vitamin E:
It is a vital link in optimal immune system functioning and it can enhance resistance to disease. The immune system identifies foreign substances and defends the body against their attack and recognises and destroys pre-cancerous and tumour forming cells. A deficiency leads to depressed antibody production and impaired immune response, also because vitamin E modulates synthesis of prostaglandin, which are important regulators of immune responses.
In cancer prevention it plays a role as a free radical scavenger and inhibits e.g. the conversion of nitrites to nitrosamines, which are strong tumour promoters, in addition to its described influence on immune reaction.
Vitamin E produces a small but significant reduction of platelet aggregation as well as it retards oxidation of LDL, implicated to be an early step in the development of atherosclerosis, so that it plays a role in prevention of coronary heart diseases and has a beneficial effect in patients with atherosclerosis and other vascular diseases.
Tocopherol works against air pollutants (mainly ozone and nitrogen dioxide) and their generated free radicals, respectively, by trapping and neutralising these radicals and consequently minimising the danger of lung damage thus protecting body tissues from pollution. It also plays a protective role against free radical accumulation associated with physical exercise, resulting in a decreased breath pentane excretion.
It also has a central role in maintaining the function and structure of the human nervous system. A deficiency initiates and perpetuates a progressive neuromuscular degeneration, which has irreversible neurological consequences if treatment is delayed.
Studies suggest that vitamin E supplementation also delays the onset of an opacity in the crystalline lens of the eye, which is a major cause of impaired vision and blindness in elderly, by slowing down the light-induced lipid peroxidation of the lens.

Skin is the outermost layer of protection to the body and it is especially vulnerable to damaging free radicals. The epidermis of the skin already contains a variety of antioxidants, but it should be possible to support the reduction of skin damage by use of agents that inhibit lipid peroxidation.

Concerning the ageing process, which involves damage of body cells by free radicals and subsequent pathological changes that slowly impair functions and eventually cause death, vitamin E terminates the radical chain reactions and confines damage to limited areas. It would be anticipated that increasing the level of protective substances, such as antioxidants, would tend to slow down the ageing process and thus lead to an extension of the normal life span.

From numerous publications it may be concluded that the toxicity of vitamin E, unlike other lipid-soluble vitamins, is very low. It has been demonstrated in animal experiments that vitamin E has neither mutagenic, teratogenic nor carcinogenic properties. Based on studies in humans, a daily dosage of 100-300 mg can be considered harmless from a toxicological point of view. It has been even demonstrated that large oral doses up to 3000 mg per day led to no consistent adverse effect. The reason might be that it is not stored in the liver. It should, however, be noted that oral intake of high levels of vitamin E can exacerbate the blood coagulation defect of vitamin K deficiency caused by malabsorption or anticoagulant therapy (KAPPUS & DIPLOCK, 1992; KAYDEN & TRABER, 1993).

The DGE (Deutsche Gesellschaft für Ernährung - German Society for Nutrition) has published an amount of 12 mg tocopherol per day as recommendation for human requirements, which is calculated to the daily consumption of linoleic acid. According to HARTFIEL (1995) this amount is deficient to react of different processes of oxidation in human organism, so that he suggests 36-100 mg per day in accordance with other authors, e.g. JENSEN et al. (1988b) stated that about 30 mg tocopherol/kg diet (equivalent to 2.5 mg/g PUFA) were enough to prevent the development of deficiency syndromes. A pharmacological dose would be between 200 and 2000 mg per day (PONGRACZ et al., 1995). Not even the satisfaction of requirements is covered by normal food intake. Three fourths of the diets consumed in the USA provided less than 100 % of the recommended daily allowance, especially young women are not sufficiently supplied. What is even of more concern is that the source of dietary vitamin E is almost exclusively vegetable oils. With the dietary recommendations to cut down on sources of fat in the diet, it is suspected that the numbers will even get worse (JOHNSON, 1995). The consumers would profit by the supplementation of animal feed when eating animal food (HARTFIEL, 1995).

1.4 Aim of the Study

With regard to all that was reviewed so far and in view of consumer demands of healthy food combined with interests of the industry to produce food of economic value, this research was designed to study influences of different feeding strategies on quality of the resulting meat or meat product. Special focus was set on requirements of meat components to gain a high-quality product concerning susceptibility of the lipid fraction against oxidative processes.

In co-operation with different institutes in Germany, several physical and chemical parameters of oxidation were studied, completed by evaluation of carcass parameters of the experimental animals. Those analyses carried out for the present thesis were determination of α-tocopherol contents, as an important compound retarding oxidation, partly fatty acid compositions, as the

main substrate for most oxidation processes, as well as volatile aldehydes, as the major secondary lipid oxidation products.

Feeding experiments were performed with pigs and cattle. As many studies had already focussed on α-tocopherol supplementation and its effects towards tissue contents and resulting lipid stability, it was more interesting to check the influences of common feeding techniques (e.g. a comparison between intensive and extensive feeding) as well as new components (lecithin and glycerol) and the influence of live weight towards α-tocopherol contents in pork.

For beef, on the other hand, several projects had been performed in the United States, where mainly steers of different breeds were raised with diets atypical from the European ones. In order to complete these results, bulls and steers were raised under common German feeding conditions (silage and grazing), partly with supplementation of α-tocopherol.

Subsequently, it was appealing to follow the long-time storage effect of antioxidants in meat products, such as Salami. In this case, not only α-tocopherol and fatty acid patterns were determined, but also the formation of secondary oxidation products was followed during storage. Taking into consideration that synergistic effects could arise when other (natural) antioxidants are present in the feed or were added endogenously to the meat product, also this aspect was investigated.

2 METHODS

2.1 Determination of α-Tocopherol in Animal Tissues and Serum

In recent years a variety of methods were published to determine the tocopherol isomers. For extraction of tocopherols from animal tissues a saponification is essential to release them from the tissue. This can be done either before or after the extraction step. Many authors describe a multiple extraction (CORT et al., 1983; BUTTRISS & DIPLOCK, 1984). However, time consuming, complicated sample preparations as well as sample transfer (CORT et al., 1983; RETTENMAIER & SCHÜEP, 1992) are unsatisfactory, because they require lots of time and chemicals and involve the risk of oxidation of tocopherol and sample loss. The method according to PFALZGRAF et al. (1995c) on the other hand provides a simple, efficient and robust analytical method without sample transfer, because saponification and extraction is performed in the same flask. It was determined that a single extraction step is sufficient for quantitative extraction of α-tocopherol from animal tissues - the other homologues are extracted less effectively. It was decided to determine only α-tocopherol (isomers) as it is the one homologue which has the highest biological activity and which is the predominant component in tissues.

Serum has not a tissue structure, but a completely different matrix than the other samples. Therefore several methods that were described in literature were checked for their suitability, efficiency and reproducibility to extract α-tocopherol. In general a denaturation of serum proteins is described instead of a saponification before the extraction step (NILSSON et al., 1978; SCHÜEP & RETTENMAIER, 1994), but some authors describe the use of saponification (HATAM & KAYDEN, 1979; DESAI, 1984). Denaturation is usually carried out with ethanol, though concentration and amount differ. The use of temperature as well as the amount and type of extracting solvent are also different in the publications. A multiple extraction such as described by MOTCHNIK et al. (1994) was avoided for the same reason of tocopherol loss by sample transfer and oxidation as mentioned above.

Quantification of α-tocopherol by Straight-Phase-HPLC (SP-HPLC) is nowadays the most popular method. The main advantages are the rapid determination and the possibility of covering a wide concentration range. Especially in combination with fluorescence detection the method is extremely sensitive and selective and therefore superior to UV-detection (MCMURRAY & BLANCHFLOWER, 1979; BUTTRISS & DIPLOCK, 1984). Straight-phase chromatography in comparison to reversed-phase has the advantage of a higher resolution (baseline separation) and durability and also the fact that α-tocopherol will elute before the other homologues, so that time for analysis is minimised (BOURGEOIS, 1992). An alternative would be the gas chromatographic determination after silylation (SLOVER et al., 1983; ULBERTH, 1991). A separation of the stereoisomers is only possible with a time-consuming procedure, combining chiral phase HPLC and capillary GC with a very polar column (RISS et al., 1994), but this method was not made available for this study.

Procedures

According to the method of PFALZGRAF et al. (1995c) 2 g of meat sample or 1 g of fatty tissue were finely cut and weighed accurately into a brown 50-ml-Schott® bottle. After addition of ascorbic acid as an antioxidant and methanolic potassium hydroxide solution (40 %) a stream of nitrogen was blown into the flask and the cap was tightly closed. After saponification for 40 min at a temperature of 80 °C (with manual shaking in-between) diluted ethanol was added. Extraction of α-tocopherol was carried out with n-hexane (containing butylated hydroxy toluene; BHT) by shaking. After centrifugation 50 µl of the organic extract were injected directly into the SP-HPLC with fluorescence detection (for detailed description refer to 8.1.1 and 8.1.3).

For determination of α-tocopherol in serum according to the developed method 0.1 g ascorbic acid was filled into a 2-ml-vial before adding 500 µl serum (sample thawed and mixed before) and 500 µl absolute ethanol. Nitrogen was blown carefully into the vial before closing the cap. After shaking vigorously the denaturation was improved by heating for 5 min at 80 °C. Extraction was carried out with 500 µl n-hexane (containing BHT), shaking for 5 min and centrifugation (see 8.1.2). The supernatant extract was injected directly into the SP-HPLC.

Identification and quantification were carried out by using external standards of α-tocopherol (see 8.1.3). The calculation was based on the External Standard method using area counts and linear regression for a wide concentration range.

2.2 Determination of Fatty Acid Patterns in Animal Tissues and Serum

In order to determine fatty acid patterns for total fat, phospholipid fraction and serum lipid fractions the fat must be extracted first. Because of the different polarities of the fat fractions, most authors apply a mixture of chloroform or dichloromethane with methanol based on the method by FOLCH et al. (1957) and n-hexane for quantitative extraction. The use of an ultra-turrax and sonification is thus promoting the damage of tissue structure and the release of fat. This of course does not count for serum samples; they are extracted by plain shaking.
For the isolation of the phospholipid fraction from the total fat the use of two aminopropyl (KALUZNY et al., 1985) or a silica column (CHRISTIE, 1985) is described. The method of BLUNK & STEINHART (1990) however was the faster and more reliable choice. However, this method can not be applied for the fractionation of serum fat, as the separation is not complete. Instead the method by FRITSCHE et al. (1998) was performed, using an aminopropyl column and three fractionation steps. The advantage of this method is the low consumption of chemicals, columns and time.

Procedures

According to an adapted method based on BLUNK & STEINHART (1990) 5 g muscle tissue or 2 g liver were extracted three times with dichloromethane/methanol and once with n-hexane using an ultra-turrax and sonification. The combined extracts are evaporated and resolved in dichloromethane (saturated with water).

An aliquot of this extract was used to determine total fatty acid pattern: Dichloromethane was evaporated, n-hexane (containing BHT) was used to resolve the fat. Fatty acid methyl esters are generated by addition of methanolic potassium hydroxide solution (11 %), shaking and neutralisation after 10 min. The hexane layer was injected directly onto the GC. For detailed descriptions see chapters 8.2.1 and 8.2.4.

For kidney fat it was not necessary to perform an extraction step, as the fat was easily accessible by just melting it out of the tissue. 200 mg of the released fat were transformed to fatty acid methyl esters (see 8.2.2). After dilution the hexane layer was injected onto the GC. As there are hardly any phospholipids in kidney fat compared to the high amount of triglycerides, the following procedure of isolating the phospholipid fraction was left out for these samples.
For the fatty acid pattern of phospholipids an aliquot of the dichloromethane extract must be fractionated on a silica solid-phase-extraction (SPE) column (BLUNK & STEINHART, 1990). After conditioning the column with n-hexane and dichloromethane (saturated with water) the extract was pipetted onto the column. Washing and elution of neutral lipids was carried out with dichloromethane and diethylether/n-hexane. The fraction of phospholipids was eluted afterwards with methanol. Fatty acid methyl esters were generated by addition of methanolic potassium hydroxide solution (11 %), shaking and neutralisation after 10 min. Extraction was carried out with 1.0 ml n-hexane (containing BHT). The hexane layer was injected directly onto the GC (see 8.2.1).

For serum fatty acid patterns a multiple extraction with dichloromethane/methanol was carried out. The extracts were dried with sodium sulphate and collected. After addition of BHT (in methanol) the combined extracts were evaporated and resolved in n-hexane. Fractionation was carried out on an amino-SPE column. After conditioning the column with n-hexane the extract was pipetted onto the column. Elution of cholesterol esters was carried out with n-hexane/dichloromethane, for elution of triglycerides, diethylether/n-hexane was used and the fraction of phospholipids was eluted afterwards with methanol. The solvent of all fractions was removed by a nitrogen stream. After resolving in hexane, fatty acid methyl esters of triglyceride and phospholipid fractions were generated by addition of methanolic potassium hydroxide solution (see above). The hexane layer was transferred into a vial and concentrated for GC-analysis. The cholesterol ester fraction was resolved in methanol/toluene and derivatised with acetyl chloride for one hour at a temperature of 100 °C. After neutralisation with sodium carbonate, the organic layer was also transferred into a vial and concentrated for injection onto the GC (see 8.2.3).

Identification and quantification in general were carried out by using external standards of fatty acid methyl esters (see 8.4). The calculation was based on the External Standard method using area counts and response factors. Rare fatty acids were also identified and quantified by external standards. The response factors were estimated to be 1.00 in this case. The identity of unknown fatty acids was checked by retention time and GC-MS. In case of identification, calculation was also done with an estimated response factor of 1.00.

2.3 Determination of Hexanal and Other Volatile Aldehydes in Ground Meat

In order to detect and measure lipid peroxidation, several very different methods are available. They focus either on primary or secondary oxidation products, or on both. Examples are peroxide value, anisidin value, TBARS (thiobarbituric acid reactive substances) or amount of fluorescent substances. Some of these unspecific methods cover a wide variety of compounds, sometimes including compounds that are not oxidation products. Other disadvantages are the low sensitivity and the low reproducibility as well as the risk of loosing or generating oxidation products during sample preparation or measurement procedure. Chromatographic methods are more selective (identification of single substances is possible) and more reliable.

For gas chromatographic determination the substances must be isolated beforehand. This can be done by vacuum distillation (AJUYAH et al., 1993b), simultaneous distillation-extraction according to *Likens-Nickerson*, solvent extraction or gas extraction.
With a headspace method mainly aroma relevant volatile compounds are isolated from non-volatile matrices. Therefore the sample is allowed to stand at a certain temperature in a tightly closed glass until equilibrium between sample and the atmosphere above the sample is achieved (CHARMAS et al., 1994). In case of static headspace a gas sample is taken then from the atmosphere in the vial with a syringe and injected onto the GC. A higher sensitivity though is attained by dynamic headspace, because the volatile compounds are continuously extracted from the sample by a gas stream and therefore the amount of sampled volatile compounds is increased. With the purge-and-trap technique the compounds are collected from the gas stream, either by trapping on adsorption material or in a cryo-device. Several polymers are used as adsorption material, depending on the compounds that are supposed to be analysed. Examples are Tenax (HEIKES, 1985; JOHANSSON et al., 1994; LAI et al., 1995), Florisil (RAMARATHNAM et al., 1993) and Porapak (SAKODYNSKII et al., 1974). Before gas chromatographic analysis can be performed the compounds have to be desorbed thermally or eluted with a solvent.

Methods that were published in literature were, for different reasons, not suitable to be applied to meat samples. Therefore we constructed a dynamic headspace mouth model system with mastication device (see fig. 3) based on VAN RUTH et al. (1995) and developed and calibrated a method for reproducible extraction and determination of volatile compounds from meat matrices.

Procedure
Before analysis, the Tenax adsorption tube had to be conditioned, the waterbath to be preheated to 40 °C and the closed apparatus to be flushed with nitrogen. Meat was homogenised just before analysis and 3.0 g were placed in the glass extraction flask of the apparatus. After adding the Internal Standard (IS; trans-2-hexenal) the piston was set into position and the apparatus was closed. The glass flask was moved up and down while the piston was switched on to perform screwing movements to simulate mastication. Extraction was carried out by flushing the headspace for 40 min with a nitrogen flow of 40 ml/min and trapping the volatiles in Tenax tubes (held at ambient temperature), while the vacuum controller at the outlet was set for 700 mbar.

After extraction, the Tenax adsorption tube was screwed into the Concentrate Headspace Injector System (CHIS) of the GC for thermal desorption of trapped volatile compounds. After a desorption time of 2 min the GC analysis was started on a DB-5 column (see 8.3).

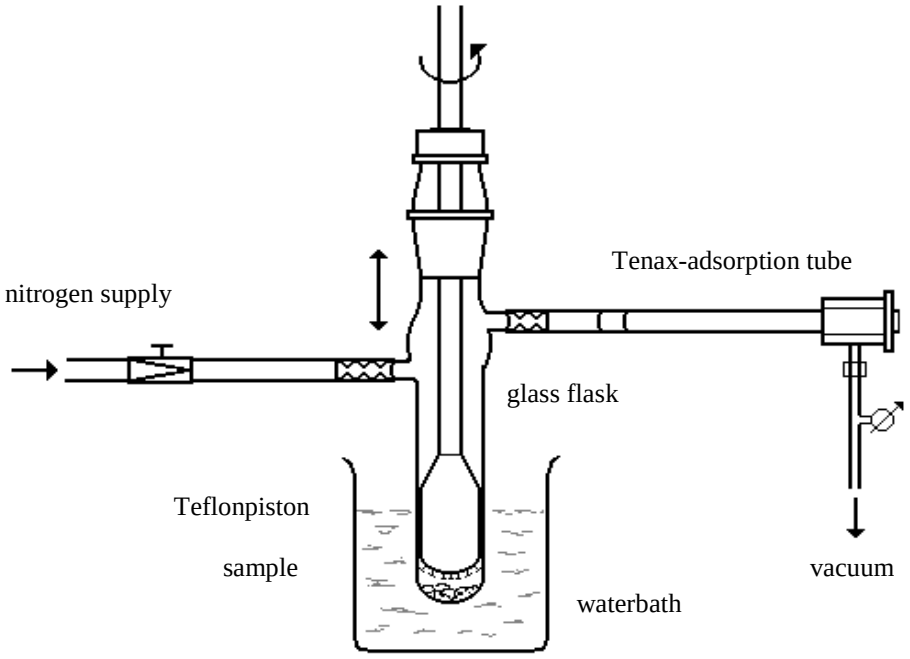

Figure 3: construction of the mouth model system for extraction of volatile compounds

Identification was carried out by using external standards of aldehydes (see 8.4). The calculation was based on the Internal Standard method using area counts of the IS and detected aldehydes. Identification of other aroma compounds was not possible as long as no standards were available, because the GC was not connected to a mass-spectrometry detector.

2.4 Statistical Evaluation

In all trials to be presented in the next chapters, a deviation occurred for results of tocopherol and fatty acid patterns when comparing individual animals and various feeding groups, respectively. These deviations can be due to either the analytical method or to the animals themselves.

Concerning the analytical methods, both α-tocopherol and fatty acid determination methods were checked for their reproducibility, linearity, precision, recovery and sensitivity and found to be accurate, even for such non-homogenous samples as salami. The deviation between animals was obviously higher than the analytical deviation, so it was decided that for almost all analyses single sampling was sufficient. Only serum and salami α-tocopherol determinations were performed in duplicate. However, for HPLC analysis of tocopherol the extract was injected in duplicate and only when the data were extremely out of range a second extraction/saponification was carried out. In GC analysis of fatty acids the injection was performed only once, the

renunciation of a second injection was mainly due to lack of time. Hexanal contents were always measured from two separate samplings.

For duplicate analyses of individual samples mean values were calculated. In case of α-tocopherol contents and fatty acid patterns only mean values and standard deviations of feeding groups are listed in the appendix, otherwise it would become too extensive. For more detailed information concerning the statistical methods used refer to appendix 8.5.

Statistical significance of differences in α-tocopherol contents between treatment means was determined by the T-Tests using the EXCEL 5.0 software programme. Different mean values are indicated in the tables with different superscripts. Small letters indicate differences in a row, and capital letters refer to differences within a column.

All data of the beef experiments were additionally subjected to Multivariate Data Analysis, using *The Unscrambler´*s regression method PLSR2 (Partial Least Squares Regression), which models both the X- and Y-matrices simultaneously to find the latent variables in X that will predict the latent variables in Y the best. The measured variables were used as X-data and the design variables as Y-data and all were weighed by 1/standard deviation.

3 PORK QUALITY

Pig meat products have been associated with an unhealthy image due to the relative proportions of PUFA and SFA. Consequently, a modification of the fatty acid composition of livestock by dietary means is receiving increasing attention, not only because of the probable relationship between fatty acid intake, plasma cholesterol and heart disease, but also because of the effect of fatty acid profile on the oxidative stability of animal tissues. On the other hand, increasing the unsaturated fatty acid content of the fat may incur problems for the meat processor and retailer in form of soft carcass fat (MONAHAN et al., 1992; MORGAN et al., 1992).

Dietary fats are widely used for fattening pigs for different reasons. As they can be digested and used energetically by pigs also in larger quantities, they are a cheap supply for energy, especially when they are backlog of production processes of other products. Secondly certain fats are fed within the scope of feeding programs for improving meat quality (FISCHER et al., 1990). In this case it is fortunate for pig-meat industry that in pigs dietary fat can be incorporated directly into body fat. Already in the 1920´s ELLIS & ISBELL (1926) had studied the influence of character and quantity of dietary fat to the composition of the body fat of pigs. They found that rations low in fat produced firm body fats while those high in unsaturated oils produced fats with the characteristics of the ingested fat. This evidence indicated that pigs tend to deposit ingested fat in preference to synthesising new fat when there is a surplus of food materials and the ingested fat is not needed for energy requirements.
The fat content has a promoting effect on vitamin E absorption, as the metabolic mechanisms are equivalent, but there are other investigations that deny that. Oxidised fatty acids or fatty acids with a high risk of oxidation can lead to a destruction or to a reduced absorption of vitamin E. Supplemented α-tocopheryl acetate is absorbed in a higher ratio than native vitamin E, because the latter is incorporated into plant components (FLACHOWSKY et al., 1997).

The two present experiments were designed to study possible changes in livestock production parameters and fatty acid profiles of the carcass fat, in relation to different feeding intensities or various dietary sources of fat. Within these distinct experiments the aim of this study was the investigation of the impact of feeding intensity as well as the use of plant lecithins and glycerol on α-tocopherol contents in muscle tissue (MLD) for pigs of different finishing sizes.

3.1 Experiment I: Influence of Feeding Intensity and Final Live Weight

The reduction of feeding intensity can be achieved in two different ways: an *ad libitum* feeding with reduced energy content and the more commonly applied restrictive (rationed) feeding, which is, in contrary to the first, avoiding a fattening of carcasses. These two types result in differences in product quality, among other things especially the *ad libitum* extensive feeding is causing a lower content of intramuscular fat and a deterioration of fat consistency and meat tenderness (KREUZER, 1993).

3.1.1 Experimental Design

Forty-eight castrated male pigs (barrows) of 25 kg live weight (Duroc x German Landrace; six per treatment, see tab. 1) were fed in three feeding periods (starter, middle and finisher diet) either intensively (13.0 MJ ME/kg) or extensively (11.5 MJ ME/kg) up to live weights (LW) of 100, 120, 140 or 160 kg. The pigs were allotted to pens in groups of six animals each.

Table 1: Experimental design for pigs "Feeding Intensity"
(Int = Intensive, Ext = Extensive, 100 - 120 - 140 - 160 referring to live weights, n = number of animals)

	100 kg LW	120 kg LW	140 kg LW	160 kg LW
intensive 13.0 MJ ME/kg	group Int 100 (n = 6)	group Int 120 (n = 6)	group Int 140 (n = 6)	group Int 160 (n = 6)
extensive 11.5 MJ ME/kg	group Ext 100 (n = 6)	group Ext 120 (n = 6)	group Ext 140 (n = 6)	group Ext 160 (n = 6)

All animals received a pelleted feed mixture of grains and soya with an additional 1.5 % of rapeseed oil to avoid dusting. The different energy contents were obtained by the ratios of the single ingredients: the feed with the higher energy content was based mainly on wheat. For the energy restricted diet, wheat was replaced by barley and wheat bran. The ratio of energy to raw protein nevertheless was calculated to be equal. The feed included a pre-mix, which covered the daily requirements of trace elements, amino acids and vitamins. The intensive diet was offered *ad libitum*, the extensive diet adjusted to the uptake of the intensive-fed group, and daily intake was recorded. The average live weight gain was slightly higher for the intensive feeding groups. The rations for all animals contained about 30 mg tocopherol per kg dry matter (due to the pre-mix), which is in accordance with the requirements and recommendations, but no additional supplementation in this trial.

Animals were raised and kindly supplied by the Department of Animal Nutrition of the University of Kassel (Witzenhausen, Germany). For further information concerning experimental design and feed refer to KUHN (1997).

3.1.2 Slaughter and Sampling Procedures

The pigs were slaughtered within the feeding groups according to standard commercial procedures (electrical stunning and exsanguination) after 16 hours without feed. Directly after slaughter, liver, heart, brain as well as chops (*Musculus longissimus dorsi* - MLD) and adipose tissue samples from the 13./14. rib were removed from the carcasses. For examination of α-tocopherol content only MLD was used, which had been ground, frozen and stored at -20 °C until needed for analysis. α-Tocopherol contents were determined once for every single animal.

3.1.3 Results from the Co-operating Institute

The parameters measured at the University of Kassel showed that, due to the different energy levels, the extensively fed group revealed a prolonged fattening period and an increased feed conversion per kg gain. In summary the carcasses of the extensively fed animals were obviously minor adiposed thus yielding much higher lean meat content. The minor energetic supply resulted in a diminished *de-novo* fat synthesis. This was the reason for a significantly higher amount of PUFA in the body fat. The PUFA content was also increasing with life weight, especially in the phospholipid fraction. Because of the soft body fat consistence the process quality of the pig meat was diminished (KUHN, 1997).

3.1.4 Results and Discussion of α-Tocopherol Contents

In the group of intensive feeding (13.0 MJ ME/kg) average α-tocopherol contents of 2.1 mg/kg (LW 100 kg), 1.9 mg/kg (LW 120 kg), 2.8 mg/kg (LW 140 kg) and 3.2 mg/kg (LW 160 kg) were determined. In the groups of extensive feeding (11.5 MJ ME/kg) 2.2 mg/kg, 2.2 mg/kg, 2.6 mg/kg and 3.1 mg/kg were determined for live weights of 100, 120, 140 and 160 kg, respectively (see fig. 4; also refer to appendix 8.6.1). Consequently, no significant differences could be detected between intensive and extensive feeding, but a correlation between live weight and α-tocopherol content was observed.

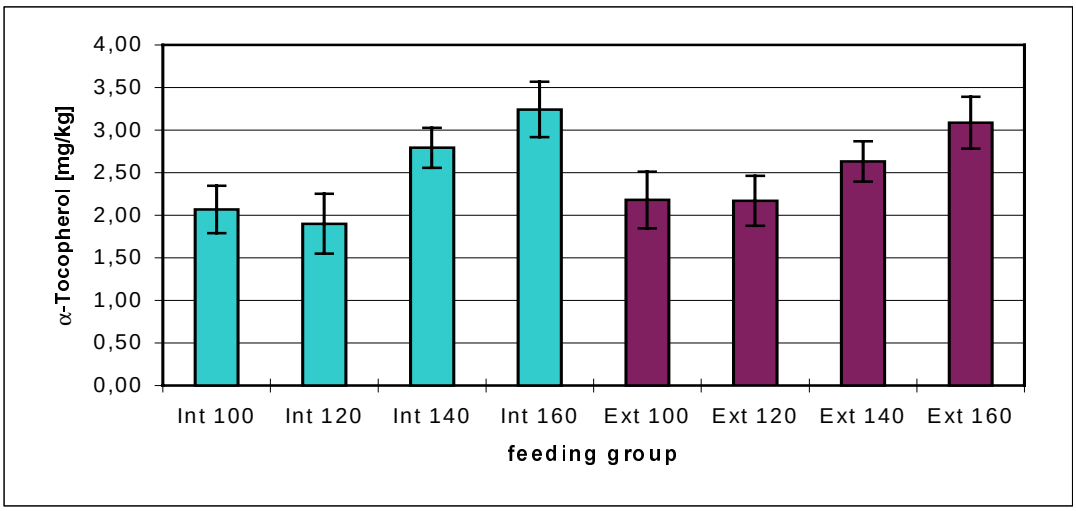

Figure 4: Pigs "Feeding Intensity" - α-Tocopherol contents in MLD, depending on feeding intensity and live weight (Int = Intensive, Ext = Extensive, 100 - 120 - 140 - 160 referring to live weights, n = 5-6)

This result differs from the results of a previous feeding experiment, which is the only one in literature to be comparable in some aspects (PFALZGRAF et al., 1995d). In that trial also barrows (castrated male pigs) were divided into an intensive and an extensive feeding group and fed until they reached life weights of about 160 kg. The feed with the higher energy content (13 MJ ME/kg) though was different: it was based on a maize, wheat, barley, and soya meal mixture.

For the energy restricted but high-fibre diet (11.5 MJ ME/kg), maize and wheat were replaced by wheat bran and turnip chips. The α-tocopherol supplementation was 30 mg α-tocopheryl acetate/kg feed in both groups.
In that experiment the α-tocopherol contents of the muscle tissues were reduced by feeding the energy restricted diet (1.4 mg/kg). It was significantly lower ($p < 0.05$) than in the group fed the higher energy level (2.0 mg/kg). In this first experiment, the α-tocopherol levels were higher in general, but also the α-tocopherol content was slightly lower for extensive feeding for 140 and 160 kg (2.6 and 3.1 mg/kg, respectively) compared to intensive feeding (2.8 and 3.2 mg/kg). But up to 120 kg live weight it was vice versa. Significance between the two feeding intensities could therefore not be shown.
The lower contents in the energy restricted group supported the hypothesis that the higher content of dietary fibre in the feed delayed the fat absorption of the animals (KUHN et al., 1994), whereas, due to the *de-novo* synthesis of fatty acids from carbohydrates, the fat content was less affected. This is equivalent to this trial: similar, rather low contents of fat (2.0-2.3 % and 2.1-2.4 % for intensive and extensive feeding groups, respectively) were determined at the University of Kassel. A correlation between α-tocopherol content and intramuscular fat content could therefore not be shown, as the fat content was independent of live weight.

Many studies have investigated the effect of a vitamin E supplementation of animal diets on the meat α-tocopherol content. But live weights at slaughter are normally within the range of 90-105 kg and this trial was the first that gave information about a correlation to staggered live weights above this limit.
The highest value for MLD (3.2 mg α-tocopherol/kg) of a non-supplemented feeding group was reported by MONAHAN et al. (1992) for pigs of a slaughter weight of 84 kg. In this case the basal feed consisted of barley, wheat, and soya bean meal with an additional 3 % of beef tallow (resulting in an α-tocopherol content of 10-50 mg). Similarly PFALZGRAF et al. (1995b) determined 2.8 mg/kg for pigs slaughtered at 95 kg and fed a diet consisting of barley and soya bean meal with an addition of 3 % soya oil, together with α-tocopheryl acetate at a basal (20 mg) level.
Other publications reported 2.5 mg/kg (PFALZGRAF et al., 1995a), 2.4 mg/kg (LEONHARDT et al., 1997) or 2.0 mg/kg (MORRISSEY et al., 1996). This last trial showed the same level as was determined in this experiment: 2.0 and 2.1 mg/kg for the intensive and extensive feeding groups. But there are also other authors that have found α-tocopherol levels below this value, e.g. 1.0-1.7 mg/kg (LANARI et al., 1995), 1.2-1.5 mg/kg (MONAHAN et al., 1990b), 0.5-0.8 mg/kg (O´SULLIVAN et al., 1997), 0.5 mg/kg (ASGHAR et al., 1991) or even the unexpected low content of 0.2 mg/kg (CANNON et al., 1996). As can be seen from these data α-tocopherol incorporation occurred within a very wide range. These variations were influenced to a high extent by the individuality of the animal itself, as could be seen from the deviation, which amounted to 10-20 % of the mean value (in this trial as well as the comparable ones found in literature). But also differences in breed and sex along with feed composition contributed to this range of results. However, significant differences ($p < 0.05$) also resulted depending on live weights: the increase of α-tocopherol contents in both feeding groups correlated positively with the increase in live weight, but not between 100 kg and 120 kg. It was expected that the α-tocopherol content in muscle tissue would increase with age, as other studies already showed that the deposition curve

increases asymptotically up to a certain maximum value (ROSENBAUER et al., 1996). It was also described already that the tocopherol uptake is improved when higher contents of fat are digested at the same time, so that a higher fat content in the feed should result in elevated α-tocopherol absorption. Concerning the influence of individual fatty components, MONAHAN et al. (1992) e.g. have claimed that increasing the linoleic acid intake of rats could result in decreased absorption of α-tocopherol. On the contrary, HENGELSBERG et al. (1993) found out that an increasing supply with linoleic acid was without influence on tocopherol adsorption.

The differences in feed composition were obviously also the reason for the different levels of α-tocopherol comparing this experiment I with the previous one (PFALZGRAF et al., 1995d), as all other parameters were the same and α-tocopherol levels in the feed were comparable. In order to get more information about the influence of various fatty components a second trial was designed.

3.2 Experiment II: Influence of Different Fat Sources and Final Live Weight

Rapeseed is attracting more interest as a pig-feeding component as it is becoming increasingly available in the form of extruded rapeseed. The cultivation of rapeseed was highly expanded in Central Europe within the last years so that it is expected that in the future rapeseed, due to its high fat and protein content, will also be employed directly in livestock production (FLACHOWSKY et al., 1994). Yet there is only little information available about use in feeding, so that three products were investigated in this trial.

Rapeseed oil contains considerable amounts of MUFA and PUFA. In pigs, the ingested PUFA are incorporated in body fat in the same proportion as present in the diet. The amount of PUFA in the feeding ration of pigs, however, is limited by the unfavourable effects on fat quality: reduced consistency and stability during storage. Another side effect is a discolouration of the fat (WARNANTS et al., 1995).
According to COORS (1990), rapeseed oil is an α/γ-tocopherol containing oil with about 600 mg tocopherols/kg, thereof ca. 35 % α- and 65 % γ-tocopherol. Soya oil is a γ/δ-oil with a total of about 1000 mg tocopherols/kg, divided up into 65 % γ-, 30 % δ- and only about 5 % α-tocopherol.

Lecithins (phospholipids, e.g. phosphatidyl choline) are mainly found in soya beans, sunflower seeds and rapeseed, but soya lecithin is by far the most important, as it is extracted commercially from soya beans. Soya lecithin is a mixture of different phospholipids, tocopherols and unsaturated fatty acids, which is an ideal combination from the nutritional point of view. The fat content of native lecithins ranges from 35 to 40 %, whereas deoiled lecithins contain only about 3 % neutral fats. It has potential use as an exogenous emulsifier to enhance the utilisation of dietary fat. Addition of lecithin improves the nutrient and energy digestibility of conventional feed, especially for piglets. This is due to the emulsifying properties (in general and in the duodenum): lecithins have an effect on digestive processes, e.g. hydrolytic cleavage of fat and proteins is supported and absorption of digestion end-products and vitamins is improved. In

addition, lecithin can serve as a highly digestible energy source in animal feeds by itself. Additionally, anabolic metabolism is stimulated as well as life weight gain and feed conversion are improved (ØVERLAND et al., 1993; GÜNTHER, 1994 and 1995). Lecithin as natural product is permitted by regulations concerning additives in animal feeds of the European Union ("Richtlinie des Rates über Zusatzstoffe in der Tierernährung" (70/524/EWG)) to be added without restriction for all animal types and feeds. In the USA lecithin has the GRAS-status (Generally Recognised As Safe) (HERTRAMPF, 1994).

Agrochemistry applied to vegetable oils is generating new potential feedstuffs for farm animals: glycerol is issued from transesterification of rapeseed oil. It is easily absorbed in the small intestine of monogastric animals. Incorporation into pig meat increases osmotic pressure, markedly decreases water loss during cooking because of better water holding capacity and as a consequence increases the meat quality and processing value (MOUROT et al., 1994).

3.2.1 Experimental Design

Sixty-four barrows of 25 kg live weight (Duroc x German Landrace; five or six per treatment, see tab. 2) were allotted to pens in groups of eight animals each. They were fed a basic feed consisting of a mixture of grains (wheat and barley) and soya bean meal with an additional 0.5 % of rapeseed oil (to avoid dust) up to live weights (LW) of 100, 130 or 160 kg. Secondary variations were obtained by addition of one of the following different fat components: 3.5 % of rapeseed oil (RO), 4 % of rapeseed lecithin (RL), 4.4 % soya lecithin (SL), or 10 % rapeseed glycerol (RG).

Table 2: Experimental design for pigs "Fat Source" (RO = rapeseed oil, RL = rapeseed lecithin, SL = soya lecithin, RG = rapeseed glycerol, 100 - 130 - 160 referring to live weights, n = number of animals)

	100 kg LW	130 kg LW	140 kg LW
rapeseed oil	group RO 100 (n = 6)	group RO 130 (n = 5)	group RO 140 (n = 5)
rapeseed lecithin	group RL 100 (n = 6)	group RL 130 (n = 5)	group RL 160 (n = 5)
soya lecithin	group SL 100 (n = 6)	group SL 130 (n = 5)	group SL 160 (n = 5)
rapeseed glycerol	group RG 100 (n = 6)	group RG 130 (n = 5)	group RG 140 (n = 5)

The fatty acid compositions of the four feed mixes were rather different. This fact also mirrors in the amount of SFA, MUFA and PUFA, as shown in table 3. The feeds were nevertheless isoenergetic (13.0 MJ ME/kg) by variation of the ingredients and covered the daily requirements of trace elements, amino acids and vitamins. The diets were offered *ad libitum* and daily intake was recorded. The rations for all animals contained about 30 mg tocopherol per kg dry matter due to the pre-mix of trace elements and vitamins. There was also no additional supplementation of tocopherol in this feeding experiment.

Table 3: Composition of selected fatty acids of different feed mixes (in % of total fatty acids; RO = rapeseed oil, RL = rapeseed lecithin, SL = soya lecithin, RG = rapeseed glycerol)

Fatty Acid	RO	RL	SL	RG
C 14:0	0,12	0,14	0,14	<0,01
C 16:0	9,14	10,91	16,66	12,26
C 16:1	0,26	0,27	0,16	0,1
C 18:0	1,92	1,87	3,46	2,17
C 18:1 c9	44,71	39,97	17,31	24,83
C 18:2 c9	29,31	36,02	49,69	43,76
C 18:3 c9	6,40	6,18	6,76	5,67
C 20:0	0,52	0,49	0,27	0,31
C 20:1 c11	1,16	0,50	0,57	0,68
C 22:0	0,40	0,28	0,58	0,27
C 22:1 c13	0,30	0,25	0,28	0,12
C 22:4 c7	0,17	0,15	0,26	0,26
C 22:6 c4	0,38	0,26	0,39	0,16
C 24:0	0,42	0,23	0,17	0,12
SFA	12,52	13,92	21,28	15,13
MUFA	46,43	40,99	18,32	25,73
PUFA	36,26	42,61	57,10	49,86

Animals were raised and kindly supplied by the Department of Animal Nutrition of the University of Kassel (Witzenhausen, Germany). For further information concerning experimental design and feed refer to KUHN (1997).

3.2.2 Slaughter and Sampling Procedures

The pigs were slaughtered within the feeding groups according to standard commercial procedures after 16 hours without feed. Directly after slaughter, liver, heart, brain and chops (*Musculus longissimus dorsi* - MLD) and adipose tissue samples from the 13./14. rib were removed from the carcasses. For examination of α-tocopherol content only MLD was used, which had been ground, frozen and stored at -20 °C until needed for analysis. α-Tocopherol contents were determined once for every single animal.

3.2.3 Results from the Co-operating Institute

The parameters measured at the University of Kassel showed that regarding the total trial period no significant differences on parameters like growth yield, carcass performance and quality and composition of meat were observed. The increase of body weight from 100 to 160 kg live weight was accompanied by a slightly elevated feed conversion and more adiposed carcasses with less lean meat contents. In general, the water holding capacity and the resulting grilling loss was

proportional to the life weight. The higher meat quality based on increasing intramuscular fat content accompanied by the decreasing content of PUFA in adipose tissues. Additionally, the improved process quality and stability of the fat compartments were very important effects. In contrast to the changes in the fatty acid pattern of adipose tissues, the PUFA increased in the tissues investigated (muscle, organs) with increasing life weights. This was mainly due to higher amounts of essential linoleic acid and arachidonic acid.
The feeding of native rapeseed lecithin led only to minor changes in the fatty acid pattern of adipose tissues, but the oleic acid content in muscle tissue and organs was diminished. In contrast, the use of deoiled soya lecithin resulted in an increase of PUFA, especially linoleic acid, in all adipose tissues. The increase of 20-25 % PUFA observed was the reason for an extremely unfavourable process quality of the carcass fat. Feeding glycerol diminished the intake of fatty acids significantly, thus yielding a very good process quality based on very small amounts of PUFA (KUHN, 1997).

3.2.4 Results and Discussion of α-Tocopherol Contents

In this second experiment α-tocopherol levels of 2.1-3.2 mg/kg have been observed. These findings are in agreement with those reported from the first experiment. Obvious is the clear influence of the different fat components. With an addition of soya lecithin the significantly ($p < 0.05$) lowest α-tocopherol contents were determined, rapeseed lecithin was causing slightly higher contents, rapeseed oil was leading to more elevated contents and rapeseed glycerol was yielding the highest contents of α-tocopherol in pork (see fig. 5; also refer to appendix 8.6.1).

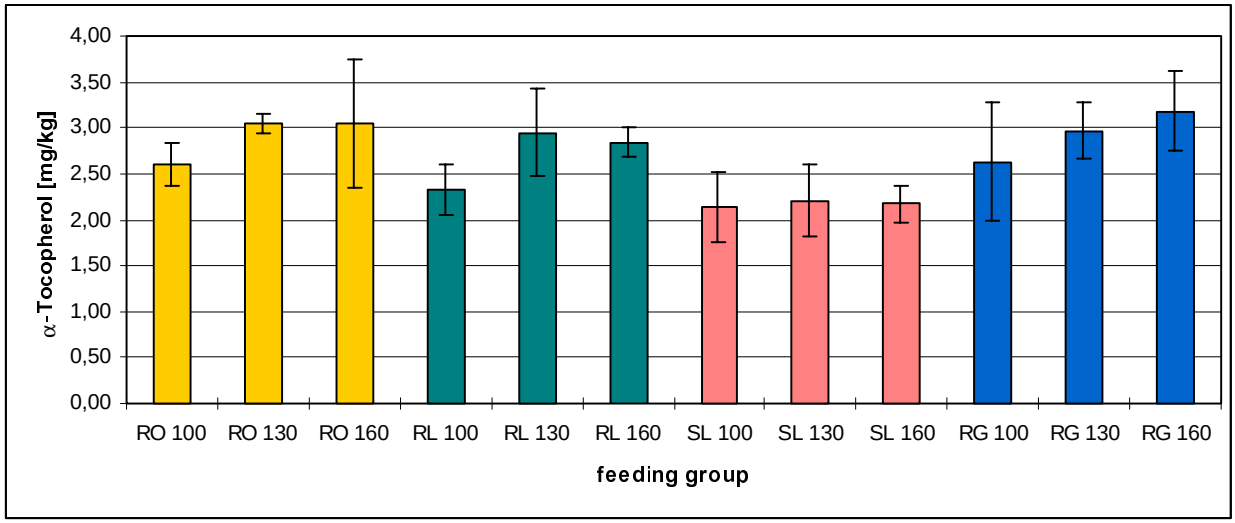

Figure 5: Pigs "Fat Source" - α-Tocopherol contents in MLD, depending on fat components and live weight
(RO = rapeseed oil, RL = rapeseed lecithin, SL = soya lecithin, RG = rapeseed glycerol,
100 - 130 - 160 referring to live weights, n = 5-6)

In the group which was fed rapeseed oil average α-tocopherol contents of 2.6 mg/kg (100 kg LW) and 3.0 mg/kg (130 and 160 kg LW) were determined. For rapeseed lecithin feeding resulted in contents of 2.3 mg/kg (100 kg LW), 2.9 mg/kg (130 kg LW) and 2.8 mg/kg (160 kg LW), whereas soya lecithin yielded only 2.1 - 2.2 mg α-tocopherol/kg for all live weights. The

last group fed with rapeseed glycerol contained 2.6 mg/kg (100 kg LW), 3.0 mg/kg (130 kg LW) and 3.2 mg/kg (160 kg LW).

There seem to be no data available of feeding experiments that have been performed especially with lecithin or glycerol in order to follow α-tocopherol contents. It should be expected that feeding with oils rich in tocopherols, especially α-tocopherol, yield meat with a high content of α-tocopherol. In that case wheat should be best, followed by sunflower oil or rapeseed, whereas soya oil and maize oil are not a good source of α-tocopherol (COORS, 1990). This seems to be confirmed by the data already mentioned: CANNON et al. (1996) found only 0.2 mg α-tocopherol/kg when pigs were fed a diet consisting of maize and soya bean meal, even with an addition of 5 % maize oil. The higher α-tocopherol levels were found for pigs that received a diet based on wheat and barley, such as described by MONAHAN et al. (1992) or LAURIDSEN et al. (2000). The latter found 3.2 mg α-tocopherol/kg for female pigs fed a basal feed of wheat and barley plus 6 % rapeseed oil. This value is very good in agreement with our results of the RO group, considering their higher rapeseed oil addition. ZANARDI et al. (2000) found also 3.4 mg α-tocopherol/kg in pigs fed an addition of 6 % sunflower oil, compared to only 1.4 mg/kg in their control group, which would confirm the theory of the α-tocopherol rich oils as the main source.

This is consequently one explanation why the group fed with additional soya lecithin had the lowest values in this study. In contrast to rapeseed oil, soya oil contains mainly γ- and δ-tocopherol. The uptake of α-tocopherol should therefore be higher for all the groups fed rapeseed products. But the α-tocopherol levels determined for all feeds were about 30 mg/kg. FLACHOWSKY et al. (1994) investigated the employment of oils rich in tocopherol (e.g. rapeseed) for dairy cattle. Besides the fact that this led to increasing α-tocopherol contents in milk, they also found that the resulting level changed depending on the source. When comparing rape seeds and the backlogs after gaining the oil they found higher α-tocopherol contents for the seeds. A possible explanation was the destruction or loss of tocopherols during processing or storage. This could also apply to this trial with the three different rapeseed products: glycerol and lecithin should then have a lower tocopherol content. But the amounts in the feed were determined to be almost the same and the glycerol-fed group did not show a lower α-tocopherol level in the meat.

LAURIDSEN et al. (2000) mentioned the possibility of a different absorption pattern. According to their latest research, the pool of α-tocopherol in the muscle fibres is related to the turnover of lipids. It was expected that the group fed rapeseed oil might show the highest levels of α-tocopherol, as this group received 4 % rapeseed oil. In fact, meat from this group contained equal amounts to the one from pigs fed the glycerol feed (with the lowest fat content). The intramuscular fat content, however, was determined to be independent of live weights and feeding (1.6-2.5 %); therefore there was no influence on tocopherol contents.
According to FLACHOWSKY et al. (1994) the different absorption could also be due to the bioavailability or uptake of α-tocopherol itself, influenced by mainly the fibres, carbohydrates or proteins. But no detailed information is published so far on this topic. It was expected that lecithins due to their emulsifying properties are especially suited to improve α-tocopherol uptake but this experiment showed that soya lecithin as well as rapeseed lecithin were not very

effective. A reason might be the statement of MONAHAN et al. (1992) that increasing linoleic acid intake could result in decreased absorption of α-tocopherol. The soya lecithin feed was by far the one, which contained highest amounts of linoleic acid (see tab. 3).

The uptake can as well be depending on other, minor ingredients that have either a synergistic or antagonistic effect. Widely discussed in this area are vitamin A (retinol) or vitamin C. HOPPE et al. (1992) stated that there was no effect of retinol on MLD α-tocopherol levels (but an adverse effect on levels in heart and liver). Similarly ANDERSON et al. (1995) could find no consistent evidence to indicate that a high level of dietary vitamin A interfered with performance or blood serum or tissue α-tocopherol concentrations in growing-finishing pigs.
Concerning vitamin C, the synergistic activity towards α-tocopherol (reducing the tocopheroxyl radical) has been shown using model systems. Direct and unambiguous evidence for its occurrence in biological systems has yet to be demonstrated (STAHL & SIES, 1996). Just CHEN & TAPPEL (1995) conducted experiments with rats combining several antioxidants and found that these combinations showed the highest protection for all tissues.

Another explanation might be the antioxidative property of α-tocopherol. According to the high amount of PUFA and especially linoleic acid in the soya lecithin diet, these pigs showed a high level of PUFA also in the meat samples. BUCKLEY et al. (1995) examined skeletal muscle from pigs fed a soya bean oil diet (5 % oil) and measured significantly higher C18:2/C18:1 ratios and a significantly higher susceptibility to iron-induced lipid peroxidation than for muscle from tallow-fed pigs. The oxidative stress therefore seemed to be high, so that α-tocopherol was needed as an antioxidant and consequently the level decreased. This might also be a reason why the α-tocopherol level of pigs fed rapeseed oil was not higher than of those fed glycerol: the PUFA content in meat from oil-fed pigs was higher.
The antioxidative property would also explain, why no differences could be detected in relation to live weights for the soya lecithin group: the higher the amounts of PUFA accumulating in the meat samples, the more α-tocopherol was consumed and a stagnation was observed.

For the other groups an increase of tocopherol contents was correlated with increasing live weights from 100 kg to 130 or 160 kg, as could be also seen in the previous experiment. Between 130 and 160 kg the difference was not significant ($p < 0.05$) anymore, because the variation within the groups was too large. Probably at this stage the maximum level was reached already. The high deviation within the feeding groups was due to individual properties of digestion and absorption. In general the α-tocopherol levels were found to be similar to those in the previously described experiment.

4 BEEF QUALITY

Shelf-life of fresh meat in a display case refers to the amount of time a retail cut can maintain a bright red, fresh appearance, before discolouration of its surface causes it to be discounted in price, minced into products of lower value or even discarded. Marketing frozen retail meat, coupled with centralised retail cutting, offers several advantages including greater marketing flexibility or lower total equipment cost, but as well as fresh beef, palatability and storage life of frozen beef are limited primarily by lipid oxidation and surface discolouration. The consumer's perception of beef quality is strongly influenced by product appearance. Colour is an extremely critical component of fresh red meat appearance, but on the other hand the necessary process of ageing, for improving tenderness in beef, leads to a decrease in colour stability. Colour has the greatest influence, among visual characteristics, on purchase decisions. Therefore, improved colour stability maximises the economic return of beef within the time period during which it is normally merchandised. Discolouration is mainly due to the oxidation of bright red oxymyoglobin to form brown metmyoglobin. Several researchers reported that lipid and pigment oxidation in fresh beef were interrelated. Thus, retarding the breakdown of lipids should result in a similar delay in metmyoglobin accumulation (LANARI et al., 1993; MORGAN et al., 1993; FAUSTMAN, 1995; LIU et al., 1995; EIKELENBOOM et al., 2000).

Several studies indicate that dietary α-tocopherol supplementation to steers causes accumulation of α-tocopherol in muscle tissue and that this antioxidant delays lipid and myoglobin oxidation and prolongs the colour stability of displayed beef (FAUSTMAN et al., 1989; ARNOLD et al., 1993b; LIU et al., 1996b; LYNCH et al., 1999). Another effect of α-tocopherol treatment is stabilised cell integrity, resulting in lower drip loss. Muscle fibre membranes from α-tocopherol-supplemented feedlot cattle are less prone to disruption during retail display of fresh beef. Nevertheless, it was also found that dietary α-tocopherol supplementation only shifted weight loss from drip to cooking loss (MITSUMOTO et al., 1995).
It is reasonable to predict that any procedure that may enhance lipid stability and stabilise beef colour, such as increasing the endogenous α-tocopherol content of beef tissues, may prolong product shelf-life and favourably impact on its economic value and image in the marketplace (MCDOWELL et al., 1996). Some authors state that measurable effects on meat colour and fat oxidation through the addition of α–tocopherol to the food can only be achieved when the levels greatly exceed requirements. Thus, titration of α-tocopherol dose and duration effects on colour and lipid stability is needed to determine minimum effective applications of this technology.

However, stabilised cell integrity and lipid stability are not only dependent on concentrations of α-tocopherol or other antioxidants, but as well on the fatty acids as components of membranes and lipids. As the population is becoming more health-conscious, not only the apparent colour is important, but also the non-visible nutrients, components and further ingredients. Beef's SFA and cholesterol content has led to a negative image of beef by some consumers, although beef provides high-quality protein and associated vitamins and minerals (RULE et al., 1997). Many consumers consequently are concerned about the contribution of beef to their total intake of

SFA. A desirable beef product should have an adequate amount of marbling, but a minimal amount of subcutaneous fat, and a high ratio of MUFA to SFA (XIE et al., 1996). Consumption of higher levels of MUFA, in conjunction with reduced levels of SFA, is desirable from a nutritional viewpoint, as it is believed to prevent increases in blood cholesterol levels and, in the case of oleate, to possibly lower blood cholesterol (BOYLSTON et al., 1995). Additionally, there is a concern in the United States, that current fish consumption (as the traditional source of nutritionally desired ω-3 fatty acids for humans, because vegetables and meat are currently poor sources) will not satisfy recommended intakes of ω-3 fatty acids. Remaining requirements must therefore either be supplied via animal products with high concentrations of ω-3 fatty acids, enrichment of foods with ω-3 fatty acids or supplementation with fish oil capsules (MANDELL et al., 1997) to reduce risk of cardiovascular diseases.

4.1 Experiment I: Influence of α-Tocopherol Supplementation for Young Bulls

Using a conventional middle European feeding system with fattening bulls, the present experiment was designed to evaluate the effects of dietary α-tocopheryl acetate dose and duration on the α-tocopherol concentration in muscle, serum, liver and kidney tissue as well as carcass and meat quality of finishing bulls. Another objective of the study was to check the fatty acid patterns with regard to a correlation to the different supplementation strategy and to the oxidative stability of the resulting beef. More details can be found in publications by SCHWARZ et al. (1998a) and AUGUSTINI et al. (1998).

4.1.1 Experimental Design

In this 2 (length of feeding period) x 3 (level of α-tocopherol supplementation) factorial experiment forty-six bulls (German Fleckvieh = Simmental bulls; ten to twelve per treatment, according to tab. 4) in the latter stages of finishing were supplemented with either 600 mg or 2000 mg α–tocopheryl acetate per animal per day for either 80 days or 120 days prior to slaughter. A total of twenty-one bulls in two control groups received the recommended daily allowance of α–tocopherol.

Table 4: Young bulls "Supplementation", divided into feeding groups
(C = control group, A = group fed 600 mg α-tocopheryl acetate, B = group fed 2000 mg α-tocopheryl acetate, 80 - 120 referring to duration of supplementation before slaughter, n = number of animals)

Supplementation	control	600 mg α-tocopheryl acetate/day	2000 mg α-tocopheryl acetate/day
80 days	group C 80 (n =11)	group A 80 (n = 11)	group B 80 (n = 11)
120 days	group C 120 (n = 10)	group A 120 (n = 12)	group B 120 (n = 12)

The bulls were allotted to pens in groups of five to six animals each, but feed intake was measured individually. They were offered maize silage *ad libitum*, with 2.6 kg concentrate per day (containing wheat, maize, soya bean meal and minerals). α-Tocopherol was added to the concentrate as α-tocopheryl acetate (Rovimix E-50, Hoffmann-La Roche, Grenzach, Germany). The control ration contained about 25 mg α–tocopherol per kg dry matter, calculated from the α-tocopherol in the total ration dry matter. The average daily dry matter intake was 8.3 kg, and there were no differences between treatments concerning performance. The animals had initial life-weights of 530 kg (120 days to slaughter) and 568 kg (80 days to slaughter) and the final overall mean life-weight at slaughtering was 641 kg.

Animals were raised and kindly supplied by the Institute of Animal Nutrition Physiology of the Technical University of München (Freising-Weihenstephan, Germany).

4.1.2 Slaughter and Sampling Procedures

The bulls were slaughtered according to standard commercial procedures at the abattoir of the company "Südfleisch", Waldkraiburg (Germany) after 20 hours without feed. Samples from the liver and the kidney fat as well as blood samples were taken directly after slaughter; the latter were centrifuged and the serum fractions were harvested and stored at -20 °C until analysis. Samples from the foreloin (*Musculus longissimus dorsi* - MLD; between the 8. and 12. rib) and the round (*Musculus semitendinosus* - MST) were removed from each carcass after 48 hours of chilling in the Institute for Meat Production and Marketing of the Federal Centre for Meat Research "Bundesanstalt für Fleischforschung" in Kulmbach (Germany). All tissue samples were vacuum-packed, frozen, stored at -20 °C until needed, and subdivided for the measurements of α–tocopherol content and determination of fatty acid patterns. α-Tocopherol contents were determined once for every single animal. For fatty acid patterns only four samples per group were chosen randomly and their tissues were analysed once.

4.1.3 Results from Co-operating Institutes

The average life-weight gain in the finishing period was irrespective of treatment. Likewise, the different levels of α-tocopherol in the diet did not influence energy intake, carcass quality and the parameters of meat quality. The intra-muscular fat contents of the MLD and MST were very low, with averages of 1.61 % and 1.14 %, corresponding to the normal values for young beef bulls. Quite large variations were found, however, between individual animals (SCHWARZ et al., 1998a). Doses of α-tocopherol above the requirements in the finishing period of young bulls had positive effects on colour stability, delayed fat oxidation and tended to reduce drip loss. Muscle tissue of the MLD of animals supplemented with α-tocopherol, especially at higher level, had a significantly higher colour stability than that of the control groups. The intensity of the redness of the meat was more stable with α-tocopherol supplementation, while the meat from control group animals turned brownish. The change in redness is directly related to the oxidation of oxymyoglobin to metmyoglobin. The increased oxymyoglobin concentration, coupled with a

decreased metmyoglobin concentration, of meat from animals given the α-tocopherol supplementation, confirms the protective action of α-tocopherol against oxidation. The oxidative stability was measured using the thiobarbituric acid method: with increasing storage time, the TBA values for the 2000 mg animals remained almost constant, while those of the 600 mg group increased slightly and those of the control groups increased significantly. A tendency of improved water retention was observed. Losses during the ageing process were not significantly different between feeding groups, but drip loss was lower in those groups that had received α-tocopherol supplementation for the longer period of time (AUGUSTINI et al., 1998). The duration of α-tocopherol supplementation tended to have a clearer effect on meat colour and oxidative stability than did the level of supplementation (SCHWARZ et al., 1998a,b).

4.1.4 Results and Discussion of α-Tocopherol Contents

In contrary to colour and oxidative stability, all tissue concentrations of α-tocopherol were significantly ($p < 0.05$; except for serum samples A 80) influenced by the dose of α-tocopherol supplementation as compared to animals receiving only basal diet. The duration on the other hand showed only an effect for the feeding groups that received 2000 mg (see fig. 6; also refer to appendix 8.7.1).

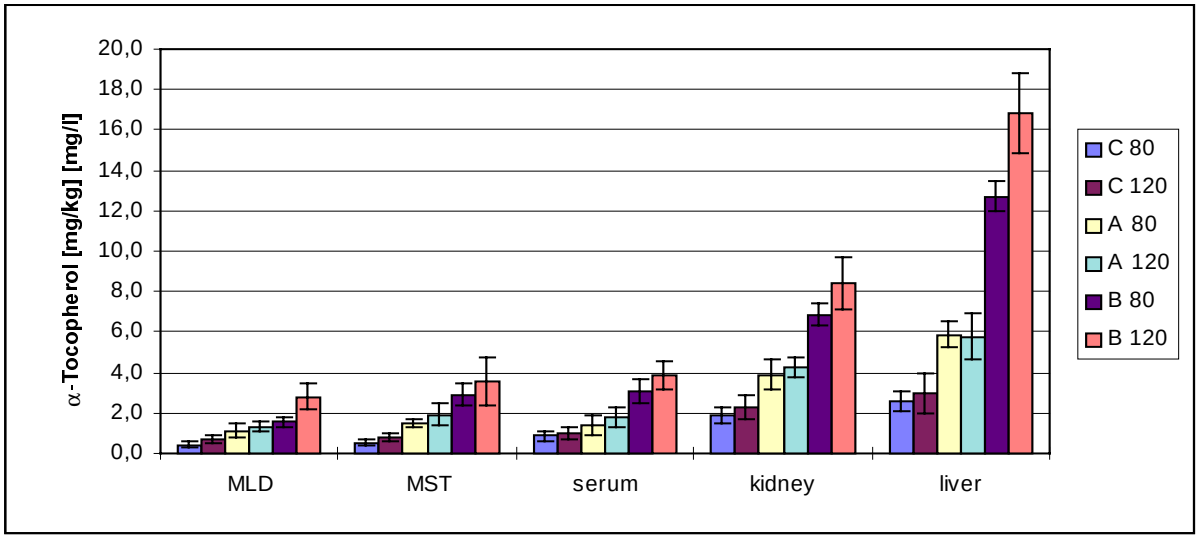

Figure 6: Young bulls "Supplementation" - α-tocopherol contents of tissues depending on feeding
(C = control group, A = group fed 600 mg α-tocopheryl acetate, B = group fed 2000 mg α-tocopheryl acetate, 80 - 120 referring to duration of supplementation before slaughter, n = 11-12)

According to expectations differences between types of tissues were obvious: for a given dose and duration liver accumulated the highest α-tocopherol contents (2.6-16.8 mg/kg), followed by kidney fat (1.9-8.4 mg/kg), serum (0.9-3.9 mg/kg), MST (0.5-3.6 mg/kg) and MLD (0.4-2.8 mg/kg). There were no significant differences ($p > 0.05$) within all types of tissues for α-tocopherol between A 80- and A 120-treatments. The influence of dose of supplementation was especially far more obvious for kidney fat and liver tissue. Control groups contained α-tocopherol contents of 2.0 and 2.8 mg/kg, respectively, feeding 600 mg for 80 and 120 days

resulted in contents of about 4.1 and 5.8 mg/kg, feeding 2000 mg for 80 days raised the contents to 6.9 and 12.7 mg/kg whereas the prolonged feeding of 120 days yielded in a further increase up to 8.4 and 16.8 mg α-tocopherol/kg, respectively.

Several authors have published results from a variety of trials (see tab. 5). Some studies have used high levels of α-tocopherol supplementation (2000-3000 mg/day) with (US) conventional feeding-time strategies (for 30-100 days), while other studies have used lower levels of supplementation (300-500 mg/day) with extended duration (for 7-12 months). But also high levels of α-tocopherol (3500 mg) were fed for up to 9 months (ARNOLD et al., 1993a). In general, the greater the amount of α-tocopherol fed and/or the longer the supplementation, the higher the tissue concentration of α-tocopherol (ARNOLD et al., 1993; LIU et al., 1995). But a comparison is confounded by breed difference studies, which state that α-tocopherol assimilation and distribution in the organism seem to be influenced by breed. Additionally, there are differences also between countries in commercial production systems, including animal feeding, which might have an effect on the results (EIKELENBOOM et al., 2000), e.g. most authors report on US feedlot-type-fattening systems with steers. Concerning this, just very few trials are comparable to this present study. On top of that, some trials have been performed with a very small number of animals per group so that the reliability of the results remains questionable.

One comparable experiment was performed by NICOLAY et al. (1995), who used double muscled Belgian Blue bulls (final weight 650 kg) fed concentrate and wheat straw plus additional α-tocopherol (ca. 2200 mg/day) for 125 days vs. 280 mg/day for control. Supplemented animals had greater α-tocopherol contents in muscles (MLD and *gluteus medius*) compared to unsupplemented ones: 1.9 mg/kg and 0.8 mg/kg, respectively. These results are within the same range as the ones from the present trial (2.8 mg/kg for group B 120, 0.4/0.7 mg/kg control). A similar trial was performed by FLACHOWSKY et al. (1994), who fed “Schwarzbunte” bulls (German Holstein Friesian) on a basis of wheat and barley, but with a supplementation of either zero or 250 mg for 350 days up to a slaughter weight of 560 kg. The resulting values for MLD were 0.7 and 3.1 mg/kg. Whereas the results for the control group are comparable, the value for group B 120 shows that the long-term feeding of a lower α-tocopherol content can yield about the same content as the short-term higher supplementation. A third study with crossbred bulls was published by DEN HERTOG-MEISCHKE et al. (1997), who fed maize silage to 2 x 20 animals with an addition of zero or 2150 mg α-tocopherol/kg for 120 days up to a final weight of 614 kg. The resulting contents in MST (the only ones to be found in literature) and *longissimus lumborum* were rather high for the control group (2.3 and 2.1mg/kg, respectively) as well as for the supplemented group (4.6 and 4.4 mg/kg). The high values for the control group were proposed to be due to a relatively high natural α-tocopherol uptake from the basal feed, which was calculated to be approximately 330 mg/kg (EIKELENBOOM et al., 2000). Even higher in MLD α-tocopherol concentration were data published by LYNCH et al. (1999) for Friesian cattle that received 2000 mg α-tocopherol/kg for 50 days prior to slaughter on top of a diet based on barley and grass silage. In this case the grass silage might have played a significant role, as it contains natural α-tocopherol and other components with a presumed synergistic effect. At this point I would like to refer to chapter 4.3.4 for further discussion of this assumption.

Table 5: Beef Feeding Experiments (selection)

Literature source	breed	trial size	sex	feed	weight [kg]	supplementation [days]	supplementation [mg]	concentration α-tocopherol [mg/kg] / [mg/l] MLD	ML	GM	MSM	PM	serum	liver
Arnold et al., 1992	Holstein	6x18	st	m	454-544	266	0		0,9				2,2	
							500		3,8				2,9	
	Angus x Hereford x Simmental	2x4	st	m	487	67	0		2,0	2,4			3,0	4,4
							2000		6,2	6,3			6,4	23,8
	Holstein	2x5	st	m	650	38	0		2,2	3,1			1,8	3,7
							2000		3,5	4,8			4,5	12,7
Arnold et al., 1993a	Holstein	2x3	st	m	520	266	0		1,5				1,6	3,5
							2000		7,1				7,5	21,6
	Holstein		st	m	520	266	2420		6,7				6,1	26,8
							3510		7,6				6,7	31,3
Arnold et al., 1993b	Holstein	3x12	st	m	522	252	0		1,2	1,6			1,4	2,1
							500		4,2	5,5			4,4	13,4
							2000		6,6	8,5			7,1	25,1
	Angus x Hereford x Charolais	3x10	st	m	470	252	0		1,0	1,3			1,8	1,6
							500		4,1	5,0			5,6	10,2
							2000		7,2	8,7			8,9	25,3
Chan et al., 1996	Holstein	2x6	st	m		122	0		0,7	1,0				
							2000		6,3	8,0				
den Hertog-Meischke et al., 1997	Piemontese x Friesian-Holstein	2x20	bu	m sil, c	614	120	0 (330)		2,1			3,2		
							2150		4,4			8,3		
Flachowsky et al., 1994	Schwarzbunte	4x5	bu	w, b	560	350	0	0,7					0,8	
							250	3,1					3,7	
Garber et al., 1996	crossbred beef	5x15	st	m		125	0			2,7	2,0		1,9	8,6
							250			4,5	2,7		3,3	17,9
							500			5,0	3,0		4,1	15,3
							1000			6,1	3,8		4,9	16,2
							2000			6,9	4,0		5,2	25,2
	Holstein	4x15	st	m		145	0			3,2	1,7		1,7	8,5
							500			4,1	2,2		3,1	11,3
							1000			5,2	2,3		3,0	14,5
							2000			6,1	2,9		3,4	16,8
Hidiroglou et al., 1988	crossbred	4x6		b (s, g)		28	1000						4,6	19,9
													9,2	27,0
Lanari et al., 1993	Holstein	2x3	st	m		126	0		0,5		0,5			
							2000		5,3		6,5			
Liu et al., 1996a	Holstein	8x9	st	m	585	42	0		3,3	1,0	1,0			
							250		5,7	2,0	3,3			
							500		6,0	1,0	3,3			
							2000		8,7	4,7	6,3			
					585	126	0		4,7	1,7	2,3			
							250		6,7	3,0	5,3			
							500		7,7	4,3	5,3			
							2000		10,0	6,0	8,3			
Lynch et al., 1999	Friesian	2x5		b, g sil		50	20	2,3		3,1		4,5		
							2000	5,4		4,4		6,9		
Mitsumoto et al., 1993	Holstein	4x3	st	m	555	232	80		1,3					
							1500		6,1					
					555	252	80		1,7					
							1500		6,8					
Nicolay et al., 1995	Belgian Blue	2x3	bu	c, w straw	650	125	280	0,8		0,8			2,4	2,3
							2200	1,9		1,9			7,3	10,8
Sanders et al., 1997	Angus x Hereford x Saler	84	st	m		100	0		3,5					
							1000		5,5					
							2000		6,1					
Vega et al., 1996	Hereford cross	4x4	st	rye g		120	0	3,4				4,5		
							2000	3,6				5,6		
				c, hay		120	0	1,9				2,5		
							2000	3,6				5,3		
Vega et al., 1997	beef x Holstein-Friesian	4x4	st	g sil, c	667	120	25	3,1						
							250	4,0						

Legend:

sex:
bu = bulls
st = steers

feed:
b =barley
c = concentrate
g = grass
m = maize
s = soya
sil = silage
w = wheat

tissues:
MLD = musculus longissimus dorsi
ML = musculus lumborum
GM = gluteus medius
MSM = musculus semimembranosus
PM = psoas major

Continuing with the results from experiments with steers, ARNOLD et al. (1992) reported that Holstein steers (final weight 650 kg), fed maize with either no supplemental α-tocopherol or approximately 2000 mg/day for 38 days, showed elevations in α-tocopherol concentrations for supplemented steers relative to non-supplemented steers, e.g. *longissimus lumborum* muscle 2.2 vs. 3.5 mg/kg; *gluteus medius* 3.1 vs. 4.8 mg α-tocopherol/kg. In another experiment with crossbred steers, also fed maize with either no supplemental α-tocopherol or approximately 2000 mg/day for 67 days up to a slaughter weight of only 487 kg, the resulting values were lower in the control group, but higher in the supplemented group (*longissimus lumborum* muscle 2.0 vs. 6.2 mg α-tocopherol/kg; *gluteus medius* 2.4 vs. 6.3 mg/kg). As can be seen, these results are higher compared to the ones from the present study, especially in the control group and even with a shorter period on feed. In general other trials with comparable supplement levels of α-tocopherol found higher α-tocopherol contents in the muscle tissues (MCDOWELL et al., 1996; refer also to tab. 5) – up to 10 mg/kg in *lumborum* muscle and 8.3 mg/kg in *semimembranosus* muscle (LIU et al., 1996a)!

FAUSTMAN & CASSENS (1991) as well as GARBER et al. (1996) found some indication that breed type may have an effect on an animal's response to α-tocopherol, e.g. that beef steers had higher (serum) α-tocopherol levels, responded more at each dosage level to a supplementation and accumulated α-tocopherol more rapidly than steers of dairy breeds. That led to the thesis, that beef steers may simply be more efficient than dairy steers in accumulating available α-tocopherol in their muscle tissue. That may be a reason, why ARNOLD et al. (1993b) measured greater plasma concentrations in beef breed steers compared to the lower α-tocopherol levels of Holstein steers. But these differences may also be due to different gender and/or different feeding practices, causing higher fat contents in the carcasses (SCHWARZ et al., 1998b). According to FLACHOWSKY et al. (1994) the α-tocopherol absorption mainly takes place together with feed lipids in the micelles of the small intestine, so that with rations rich in fat higher α-tocopherol absorption is expected. This would be an explanation why α-tocopherol seems to be less well stored by Belgian Blue bulls than by Holstein steers, since meat from Belgian Blue bulls is much leaner than from Holstein steers. On the other hand, α-tocopherol concentrations in MLD from the present trial were unrelated to their lipid contents. Also LIU et al. (1994) found that no correlation occurred between tissue lipid content and α-tocopherol concentration, as well as studies vice-versa also indicated no differences among supplemented and non-supplemented steers for tissue lipid content.

Depending on a large variety of influencing factors it was concluded that the same dose of applied α-tocopherol could lead to incorporation of different contents when comparing several individuals. In this present experiment it could be very well shown (see fig. 7), that individuals differ from each other in the amount of α-tocopherol incorporated, but within one animal the different tissues show a correlation.

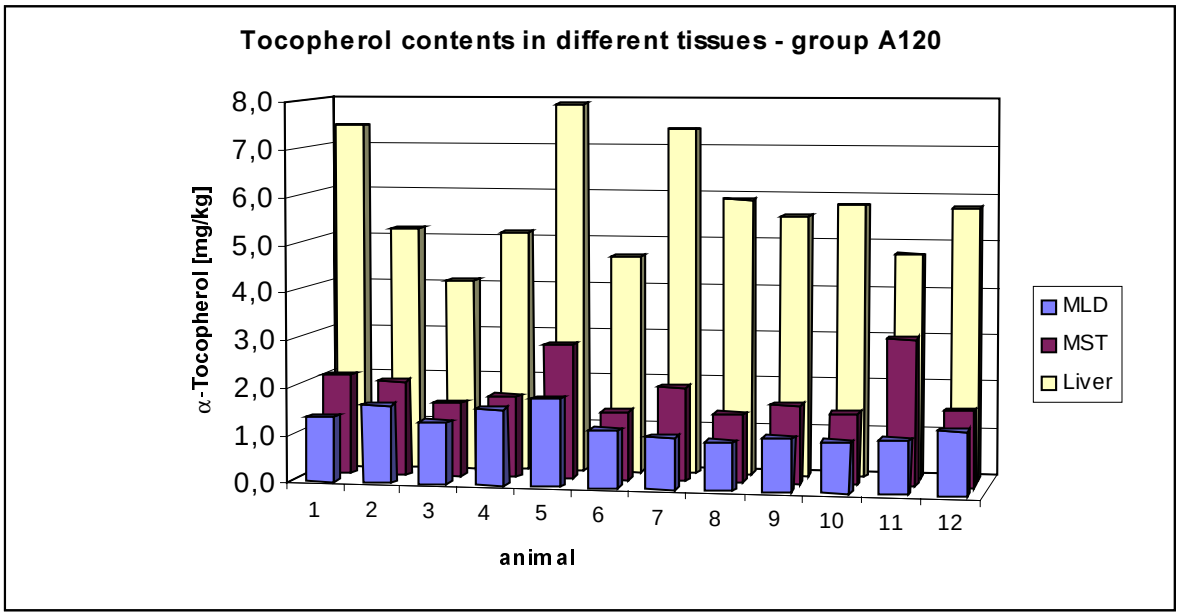

Figure 7: Young bulls "Supplementation" - α-tocopherol contents depending on individual animals

In general the duration and dose of supplementation are important as well as the source of α-tocopherol, the composition of the feedstuff, animal type and age, composition (especially fat content) of the tissues and organs, and functionality of the digestive organs. Inhibition of α-tocopherol uptake can be due to protein shortage in the feed, a surplus of vitamin A, oxidised PUFA, or disturbance in digestion. Promoting factors can be a high fat content in the diet or the presence of other antioxidative vitamins (FLACHOWSKY et al., 1997). The initial level of α-tocopherol in beef muscle before the start of supplementation, which results from the previous feeding strategy, is also an important consideration (LIU et al., 1995). Concerning the source of vitamin E it has to be paid attention to the chemical form of α-tocopherol being added, which very often is not clearly specified in the publications (as already mentioned on page 12). In comparison to the natural L-α-tocopherol, the stability of D-α-tocopherol in ruminal liquor is high; it is not metabolised or oxidised to any great extent in the gastrointestinal tract of cattle. Results suggest strongly that D-α-tocopherol or its acetate form are more available to bovine tissue than the L-epimer (HIDIROGLOU et al., 1988) and that D-α-tocopherol and its acetate esters increase plasma α-tocopherol concentrations faster than the racemic products (LIU et al., 1995).

In this present trial there were only significant differences between MLD and MST for the feeding groups with higher α-tocopherol supplementation (A 120, B 80 and B 120). Nevertheless there appeared to be a tendency for the contents of α-tocopherol in the MST to be higher than in MLD throughout all groups. For control group C 80 the difference was only 0.4 vs. 0.5 mg/kg, but for B 120 it was already 2.8 vs. 3.6 mg/kg. The highest increase, though, was found in group B 80: MLD contains 1.5 mg/kg, whereas MST has a value of 2.9 mg/kg. In general there are only slight differences between different muscle types, but ARNOLD et al. (1992, 1993a,b) found that α-tocopherol accumulation is muscle dependent. The concentration of α-tocopherol in *gluteus medius* was always greater than that in *longissimus lumborum*.

There are no clear explanations why skeletal muscles differ in α-tocopherol concentration, but possible reasons follow: ASGHAR et al. (1991) explained differences in α-tocopherol uptake by varying capacity between breeds, as they found that the average α-tocopherol content tended to be higher in certain muscle types. Different muscles consist of different proportions of red and white fibres. Red fibres contain numerous small mitochondria between myofibrils, whereas white fibres contain fewer but larger mitochondria. It has been observed before, that mitochondria have the greatest capacity for α-tocopherol storage, therefore more and smaller mitochondria may provide more membrane volume for potential retention of α-tocopherol. In addition, different fibre sizes among muscles could also affect membrane volume.
The distribution of capillaries may be another factor that affects α-tocopherol accumulation (LIU et al., 1996b). Normally, red muscle fibres have a much greater capillary blood supply than do white fibres. The residual blood entrapped within these and its associated α-tocopherol would also provide increased concentrations of α-tocopherol in red muscle (ARNOLD et al., 1993a). Unfortunately equilibration between dietary α-tocopherol intake and subcellular pools of α-tocopherol in muscle has not been studied yet.
For pigs found JENSEN et al. (1988a) and LAURIDSEN et al. (2000) that content and storage capacity of α-tocopherol was related to the oxidative capacity of the muscle (that probably inspired several researchers to choose MLD, *gluteus medius* and *psoas major* as a set of muscles varying in oxidative capacity): α-Tocopherol concentration is higher in oxidative psoas major compared to the glycolytic MLD. Their latest research also suggested that the pool of α-tocopherol in the muscle fibres is related to the turn-over of lipids, such as also SCHWARZ et al. (1998b) specified that such differences may be connected to the proportions of various types of muscle fibre and their varying ability to store α-tocopherol or the respective proportions of PUFA in the muscle. Therefore muscle fibres with a greater oxidative capacity stored more lipid and α-tocopherol.

The various organs and tissues reflect the α-tocopherol supplementation in different extent, as tissues clearly differ in their ability to accumulate and retain α-tocopherol. Adipose tissue and liver are considered to be the major storage deposits of the vitamin, presumably based on their mass and ability to accumulate the vitamin. Therefore the highest tissue accumulation of α-tocopherol was in liver, adrenal, pancreas, and spleen followed by subcutaneous fat, kidney, plasma and muscle (LIU et al., 1995; GARBER et al., 1996; MCDOWELL et al., 1996).
Tissue accumulation of α-tocopherol in supplemented animals appeared to occur in a dose- and duration-dependent manner, related to the logarithm of dietary intake (except for adipose tissue; PARKER et al., 1989). At the beginning the α-tocopherol concentration increased in blood. Then it was transported further on to the liver and stored into adipose tissues. Therefore, equilibration between tissue concentration and dietary intake was more rapid for blood and liver as well as other inner organs than for MLD (SCHAEFER et al., 1995). JENSEN et al. (1988a) suggested that the concentrations of α-tocopherol in serum and liver reflect the immediate nutritional status of the animal, whereas the α-tocopherol concentrations in adipose and skeletal muscle tissues reflect its long-term nutritional history or even indicate a long-term surplus (FLACHOWSKY et al., 1994; MORRISSEY et al., 1996). After long-term feeding of a supplemented diet the concentrations in organs and tissues are decreasing in the order inner organs (liver, heart, lung) > adipose tissues > muscle tissues > blood (MONAHAN et al., 1990b; SHEEHY et al., 1991), but

other authors also reported different results. But the dose of supplementation also influences the order in organs and tissues (ARNOLD et al., 1993a; FLACHOWSKY et al., 1997).

Concerning the order of tissue concentrations the results from this present experiment differed slightly, as serum values were at least the same level as muscle values. This may therefore be due to the relative short period of supplementation. Serum α-tocopherol values increased from 0.9 mg/l for control groups to 1.8 mg/l after feeding 600 mg/kg for 120 days, up to 3.1 mg/l and 3.9 mg/l for the groups fed 2000 mg/kg for 80 and 120 days, respectively. FLACHOWSKY et al. (1994) again found similar contents for the bulls fed 250 mg/kg for 350 days: serum contained 0.8 mg/l in the control group and the amount increased to 3.7 mg/l in the supplemented group. The Belgian Blue bulls (NICOLAY et al., 1995) contained higher levels with 7.3 mg/l serum in the supplemented group (2200 mg/kg for 125 days). This result is more comparable to the US-experiments by ARNOLD et al. (1992), who determined already 6.3 mg/l for crossbred steers supplemented with 2000 mg/kg for 67 days, CHAN et al. (1996), who found 8.0 mg/l in Holstein steers fed 2000 mg/kg for 122 days, or GARBER et al. (1996), who measured 5.0 and 6.9 mg/l in crossbred steers supplemented with 500 and 2000 mg/kg for 125 days, respectively.

Liver values in this present experiment were increasing significantly ($p < 0.05$) depending on the dose of supplementation. Control groups contained about 2.8 mg α-tocopherol/kg, feeding 600 mg/kg led to an increase up to 5.8 mg/kg, whereas the content within the 2000 mg feeding groups further raised significantly from 12.7 mg/kg to 16.8 mg/kg for durations 80 and 120 days, respectively. In this case, the Belgian Blue bulls (NICOLAY et al., 1995) showed lower results with only 2.3 mg/kg for the control group (which already received 280 mg/kg) and 10.8 mg/kg for the supplemented group. Higher were again the US-trials; e.g. ARNOLD et al. (1992) determined 4.4 and 23.8 mg/kg for control group and supplemented group, respectively. GARBER et al. (1996) found already 8.6 mg/kg in the control group and values increased up to 15.3 and 25.2 mg/kg for steers fed either 500 or 2000 mg/kg for 125 days, which were about the highest values to be published.

Liver and subcutaneous fat seem to have a certain capacity to accumulate more α-tocopherol at higher levels of supplementation, which led to a maximum α-tocopherol content of more than 30 mg/kg liver for Holstein steers fed about 3500 mg/kg for 266 days (ARNOLD et al., 1993a).
Subcutaneous fat was not examined in this trial, but kidney fat instead. The results are not directly comparable, but only few values are published concerning adipose tissue α-tocopherol concentrations and even less about kidney fat. In the present study significant increases were measured between all feeding groups except A 80 and A 120. Nevertheless the α-tocopherol concentration raised stepwise from 1.9 mg/kg in control group C 80 to 2.3 mg/kg (C 120), 3.9 mg/kg (A 80), 4.2 mg/kg (A 120), 6.9 mg/kg (B 80) and 8.4 mg/kg in group B 120. NICOLAY et al. (1995) published values for kidney (6.4 mg/kg) and subcutaneous fat (7.5 mg/kg), which are both in the range of the above-mentioned results from the present trial. A reason, why control group C 120 had higher concentration throughout all tissues could not be found. Therefore the only remaining explanation may be the individuality of the animals.

Nutritional status with respect to vitamin E is commonly estimated from plasma concentration. But it should be taken into consideration that the use of blood serum α-tocopherol concentration as an indicator of an animal's α-tocopherol status needs to be critically interpreted in each individual case. Results by ARNOLD et al. (1993a) revealed that plasma levels of α-tocopherol may not be used as a reliable indicator of muscle α-tocopherol concentration unless the plasma concentration is known to have been at a steady-state for 3 to 4 months, due to the time required for equilibration of the muscle α-tocopherol pool with dietary α-tocopheryl acetate intake. According to these findings as well as PARKER et al. (1989) the results from the present study would not be reliable, as the animals were fed only 3-4 months, which obviously cannot be the time of steady state. However, blood serum α-tocopherol concentration is most likely reflective of recent dietary intake of α-tocopherol (SCHAEFER et al., 1995; MCDOWELL et al., 1996).
On the other hand may adipose tissue tocopherol levels also not accurately reflect status since this α-tocopherol pool might not be bioavailable, i.e. in equilibrium with plasma, and thus may be more reflective of long-term dietary absorptive history than current status. These findings again illustrated the disadvantage of using total plasma α-tocopherol levels as the sole indicator of vitamin E status, particularly if not corrected for plasma lipid levels (PARKER, 1989).

Another aspect which might influence α-tocopherol concentrations in tissues is explained by NOCKELS et al. (1996), who found that plasma α-tocopherol content was reduced by stress treatment in heifers fed 1000 mg α-tocopheryl acetate/day and increased in non-supplemented α-tocopherol-deficient heifers. This would confirm that plasma α-tocopherol values might not accurately reflect body status of α-tocopherol. Stress also decreased red blood cell and liver α-tocopherol content in supplemented cattle. Possibly the loss of liver α-tocopherol content after stress was lower in the α-tocopherol-deficient heifers because most of the analysed vitamin was bound in membranes and unavailable for mobilisation. An alternative explanation may be the impaired mobilisation of α-tocopherol due to inadequate amounts of α-tocopherol binding protein. In the current study, muscle α-tocopherol values were not generally increased by supplementation, possibly because its amount or duration was insufficient. Tissue α-tocopherol concentrations were reduced by stress only when a diet adequate in α-tocopherol was fed. High density lipoprotein (HDL) may serve to remove excess tissue α-tocopherol and return it to the liver, analogous to its role in promoting free cholesterol efflux from tissues. α-Tocopherol efflux from adipose tissue, one of the major stores of α-tocopherol in the body, may be important to maintain tissue levels during vitamin E deficiency (KAYDEN & TRABER, 1993).

Dietary vitamin E requirements of cattle have not been clearly defined. The US National Research Council - Committee on Dietary Allowances, Food and Nutrition Board), made a recommendation of 15 to 60 mg/kg of diet dry matter for young cattle; no requirement was defined for adult cattle. For lactating cows a plasma α-tocopherol concentration of > 4 mg/l is estimated to be sufficient, whereas a content below 1.5 mg/l is indicating a deficiency (FLACHOWSKY et al., 1997). MCDOWELL et al. (1996) suggested 200-500 mg per animal per day for beef cattle. In studies by LIU et al. (1995), an average daily consumption of a basal 60 mg/kg did not lead to any abnormal animal health or reduced animal performance. Therefore, they concluded that as little as about 60 mg α-tocopherol/kg daily might meet the vitamin E requirement of finishing cattle. This is why they referred to their dietary supplementation as

supranutritional. Hypervitaminosis has not been investigated in cattle. Extrapolations suggest upper safe daily dosages of 75 mg/kg of body weight and maximum tolerable dosages are in the range of 1000-2000 mg/kg diet (ARNOLD et al., 1993b).

A review of the literature would suggest that an α-tocopherol concentration of 3.0-4.0 mg/kg tissue is required for improvement of oxidative stability in whole muscle and/or comminuted type beef products: stabilising beef colour by reducing accumulation of metmyoglobin and thiobarbituric acid reactive substances (TBARS) and finally extending shelf-life. (FAUSTMAN et al., 1989; ARNOLD et al., 1993a,b; SMITH et al., 1994; MCDOWELL et al., 1996). Although only group B 120 in this study reached levels of 3.6 mg/kg in MST (and MLD of B 120 and MST of B 80 were close with 2.8 and 2.9 mg/kg) and no retail experiments were performed, the positive influence on colour and lipid stability was measurable for all supplemented groups.

Supplementing cattle with α-tocopherol resulted in steaks (SANDERS et al., 1997) that exhibited superior lean colour, less surface discolouration and more desirable overall appearance during retail display than control steaks. LIU et al. (1996a,b) as well as ARNOLD et al. (1992; 1993b) also experienced that dietary α-tocopherol supplementation stabilised redness and colour saturation and effectively extended colour display life of fresh beef. The results showed that steers supplemented with α-tocopherol had greater muscle pigment stability as evidenced by higher visual colour evaluation scores. It appears that since α-tocopherol supplementation allowed a greater amount of α-tocopherol to be incorporated, retail shelf-life of α-tocopherol-supplemented cuts was increased by approximately 2-3 days when compared to cuts from non-supplemented steers (MORGAN et al., 1993).
TBARS values - which are used as an indicator of lipid oxidation - for fresh beef products from α-tocopherol-supplemented steers were lower and therefore more resistant to lipid oxidation compared to retail cuts from non-supplemented steers (MORGAN et al., 1993; EIKELENBOOM et al., 2000). Also during simulated retail display, accumulation of TBARS was greater in beef from control than from supplemented steers. However, taste panellists detected no difference (ARNOLD et al., 1992, 1993b; NICOLAY et al., 1995; LIU et al., 1996a,b). These results agree with those by FAUSTMAN et al. (1989), who concluded that when α-tocopherol is supplemented to animals during the finishing period, elevated levels of α-tocopherol are incorporated into cellular membranes where it performs its antioxidant functions to decrease the *post mortem* muscle tissue's susceptibility to pigment and lipid oxidation.

MCDOWELL et al. (1996) could show that α-tocopherol increased performance of feedlot cattle and improved average daily gain and gain/feed ratios (feed efficiency) for young cattle in a metabolically demanding state. Also increasing stress apparently increased the potential for α-tocopherol to improve cattle performance. This report is contradictory to the results from the present trial (SCHWARZ et al., 1998b) and those of many other authors who state that feeding supplemental α-tocopherol does not appear to affect average daily weight gain, energy intake or feed efficiency (ARNOLD et al., 1993b; EIKELENBOOM et al., 2000), nor does it influence feedlot performance, carcass characteristics (FLACHOWSKY et al., 1997), marbling score or USDA Quality Grade (SMITH et al., 1994; NICOLAY et al., 1995; GARBER et al., 1996; SANDERS et al., 1997).

4.1.5 Results and Discussion of Fatty Acid Patterns

Results of fatty acid patterns were not very easy to interpret, as from the first view it did not seem that there were differences related to feeding strategies. The absolute values for each fatty acid differed already within feeding groups, so that taking these high standard deviations into account, significant differences between feeding groups could not be detected anymore. For this reason the results from the different feeding groups were averaged for each tissue and those total mean values were used for comparison with data from literature.

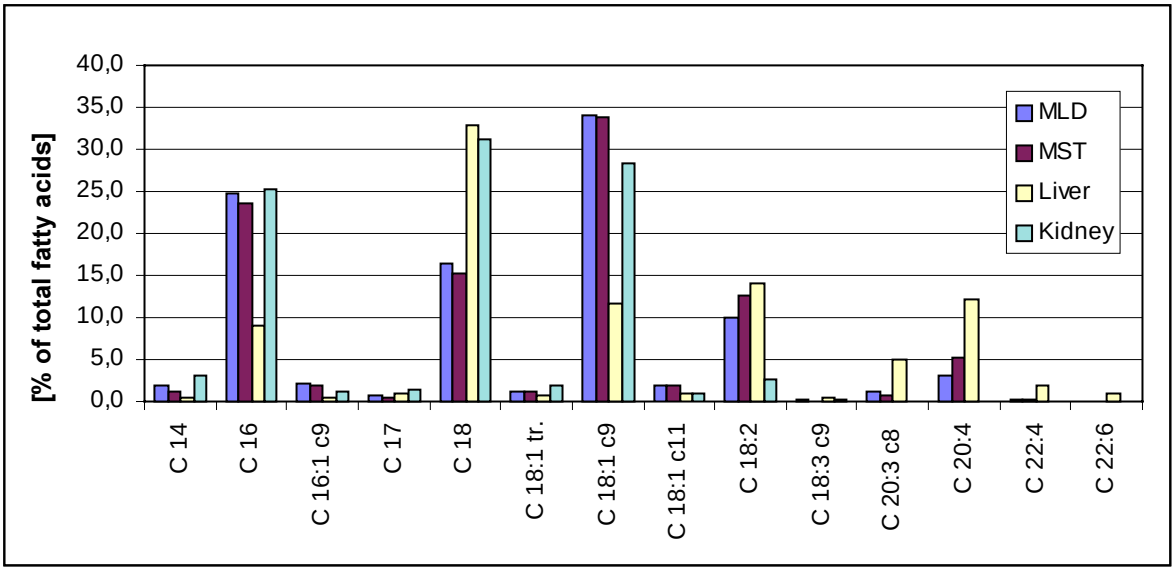

Figure 8: Young bulls "Supplementation" – selected fatty acids of total fat in different tissues

The tissues themselves showed differences from each other as expected (see fig. 8, also refer to appendix 8.7.2). In total fat of muscle tissues MLD and MST oleic acid was the major fatty acid (34 %), followed by palmitic acid (25 %/23 %), stearic acid (16 %/15 %), linoleic acid (10 %/13 %) and arachidonic acid (3 %/5 %). Further myristic, palmitoleic, elaidic, vaccenic and eicosatrienoic acid were determined in the 1-2 % range. Other PUFA could not be detected, except for 0.3 % docosatetraenoic acid. Summarised, levels of 44 % SFA, 40 % MUFA and 15 % PUFA were determined in MLD. In MST only slight differences resulted with a shift of 4 % from SFA (40 %) to PUFA (19 %).

Liver tissue contained only 12 % oleic acid and 10 % palmitic acid, but more than 30 % stearic acid and 12 % arachidonic acid. Except for linoleic acid (14 %) all other detectable fatty acids with a chain length up to C18 were below 1 %, but some long-chain and PUFA were present to a higher extent: eicosatrienoic acid (5 %), docosatetraenoic acid (2 %) and docosahexaenoic acid (1 %). This led to 44 % SFA, only 15 % MUFA but 35 % PUFA.
Kidney fat on the other hand contained less than 3 % PUFA but about 25-30 % for each palmitic acid, stearic acid and oleic acid. Myristic, palmitoleic, margaric, elaidic, vaccenic and linoleic acid were present with 1-3 %, but no long-chain fatty acids occurred. In this tissue the highest SFA content with 61 % resulted, the remaining 33 % were MUFA.

Neutral lipids are present as microscopic droplets within the muscle cell, or in adipocytes (fat cells). The neutral lipids are not essential to muscle, but they do provide fatty acids for energy metabolism in living muscle. The fatty acid composition of storage fats (adipose tissue and marbling) therefore is generally quite different from that of structural fats, being richer in SFA and MUFA with relatively small concentrations of PUFA. The neutral or nonpolar lipids contain about 40-50 % MUFA and less than 2 % of the most highly unsaturated fatty acids (ALLEN & FOEGEDING, 1981). The latter statement though needs to be qualified, as the experimental animals showed relative high contents of PUFA in total fat of MLD.

When in the following the results from this present trial are compared with other publications, it has to be kept in mind that several of those were performed with a complete different aim.
In the early 1970´s, HECKER et al. (1975) were influenced by the beef industry intending to produce low-fat beef in light of consumer demands when they studied Hereford, Angus and Holstein steers and heifers during their growth periods up to only 390 kg. They determined increases in total amount of fat (as animals mature they deposit increasing amounts of fat in the body tissues). In addition, fatty acid compositions of muscular and subcutaneous fat have been found to increase in (mono) unsaturation with growth and correspondingly decrease in saturation. In muscle (*biceps femoris*) they also found oleic acid (48 %) to be the major fatty acid, followed by palmitic acid (26.8 %). Palmitoleic, stearic, linoleic and myristic acid followed with 9.3 %, 7.7 %, 3.8 % and 2.2 %, respectively. In subcutaneous fat only slight differences to muscle tissue occurred, so that the content of SFA in both cases was 38.5 %, MUFA were ca. 58 % and PUFA only 3.5 %. It was expected that subcutaneous fat would show a similar pattern to kidney fat as both function in the body to store fat and to protect tissues and organs against shock. But both results are very different to the previously presented ones. Differences could be due to changes in mainly feeding (and breeds – among others) throughout the last 25 years, as a consequence of the desired production of low-fat beef at that time.

LARICK et al. (1989) studied fatty acid profiles of neutral lipid and phospholipid fractions with regard to fatty acids being important precursors of beef flavour. They analysed MLD of Bison, Hereford and Brahman steers of similar age (18 months) finished on identical diets (maize and soybean), as variations in bovine carcass composition (e.g. fatty acid composition) have been attributed to genetic effects (breed), sex characteristics, nutrition and physiological maturity. Different breeds of cattle, in particular, have been shown to differ in distribution of their carcass fat, e.g. Brahman steers tend to deposit more of their total fat as subcutaneous fat, whereas Angus steers have more seam fat as a percentage of carcass weight. The major fatty acids in the neutral lipid fraction of each breed, however, were oleic (40.6-44.4 %), palmitic (30.1-35.8 %) and stearic acid (12.3-17.0 %) which together accounted for 88-90 % of the total fatty acids. The other fatty acids contributing to the pattern were palmitoleic, myristic, linoleic, myristoleic and linolenic acid listed in a decreasing order with a range of 4.8-0.5 %. As a result, neutral lipids of MLD were composed of about 48-53 % SFA, 47-52 % MUFA and 1-2 % PUFA. These values were already closer to the ones from this present trial, but a difference remained in the higher values of oleic and palmitic acid, which led to higher contents of SFA and MUFA, accompanied by the low values for PUFA such as linoleic acid. A reason might still be the feeding strategy from about 10 years ago, but another explanation for the lower values for PUFA could be the

different analytical procedure, since LARICK et al. (1989) separated total lipids into neutral and polar ones, which was not done in the present trial. Total lipids contain more PUFA due to the higher content of phospholipids that is included.

The latest results were described by ITOH et al. (1999) who raised Angus and Simmental steers to particularly heavy carcass weights of 500 kg, which should provide an opportunity to investigate changes in fatty acid composition over a wide range of intramuscular fat levels (3-18 %). Animals were started on pasture and then fed a diet based on maize grain and 30 % pasture silage for 13 months. Triglyceride fatty acids in *longissimus* muscle were corrected to a constant level of intramuscular fat, because the authors proposed that a comparison would be questionable if no common levels of intramuscular fat had been chosen. Oleic acid was predominant with 41-43 % and together with palmitoleic acid (ca. 3-4 %) the MUFA content of 45 % was found to be slightly higher than in the present trial. Palmitic acid (27-30 %), stearic acid (14.5 %) and myristic acid (3-3.5 %) yield an SFA content of 45-48 %, which is also higher compared to the present study, while linoleic and linolenic acid represent PUFA with a very low content of about 1-2 %. This could be due to the fact that values were determined only for the triglyceride fraction and did not include the more unsaturated phospholipid fraction as in the present trial. Another explanation might be that ITOH et al. (1999) in triglycerides experienced a decrease of linoleic and linolenic acid with increasing level of intramuscular fat, which was very high in their study.

Rather new and very similar are also the results by RULE et al. (1997) who studied the effect of duration with regard to beef's SFA and cholesterol content. Crossbred steers were fed on a base of maize and barley for certain lengths of time after weaning. The MLD SFA (44 %) changed little with duration, as also stearic acid (14.0 %), palmitic acid (26.5-28.8 %) and myristic acid (3.7-2.6 %) remained rather stable. Oleic acid (37-45 %) increased, therefore also MUFA (46-51 %) increased, although palmitoleic acid (9.8-5.9 %) showed a decrease. PUFA (9.0-3.0 %) decreased due to declining contents of linoleic acid (6.1-3.6 %) and arachidonic acid (2.9-0.8 %). These values were closest to the results from the present trial, so that it can be assumed that in fact feeding has changed within the last years. Nevertheless there still remain minor differences, especially in PUFA, but these could be allocated to several factors. Sex and breed effects on proportions of major fatty acids in adipose tissue have been reported for several cattle breeds. Nutritional factors also affect tissue fatty acids in cattle. But in this case it could also be the fact, that the crossbred steers were younger than the bulls from the present trial.

FLACHOWSKY et al. (1994) and MANDELL et al. (1997) performed experiments to study the influence of feeding. FLACHOWSKY et al. (1994) raised Schwarzbunte bulls (dairy breed) up to a body weight of 540 kg with concentrate and wheat straw. Rapeseed supplementation (350 days with 0, 7, 14 or 21 % rapeseed) significantly enhanced the α-tocopherol concentrations in all body samples (in MLD from 0.7 to 3.1 mg/kg, in depot fat from 4.5 to 14.9 mg/kg) and increased the intramuscular fat content from 1,3 to 2% (due to the fat content of feed raising from 2.5 to 10 %). Additionally, rapeseed supplementation decreased C 16 fatty acids (palmitic 24.2-19.0 % and palmitoleic acid 2.6-1.5 %) and increased C 18 fatty acids (stearic 17.8-23.5 % and oleic acid 37.4-38.5 %; due to incorporation from rapeseed) in depot and muscle fat (MLD). Other fatty acids present were linoleic acid (6.2-5.6 %), myristic, margaric and arachidonic acid with

ca. 1-2 % each as well as lauric and linolenic acid < 1 %. Muscle fat contained significantly more MUFA and PUFA (40.2 % and 7.4 %) than depot fat (33.5 % and 2.0 %, respectively). These values, especially the ones from the group fed no rapeseed, were very close to the results from the present trial. Just the contents of PUFA, e.g. linoleic and arachidonic acid, were lower. Same as in the present trial, differences between feeding groups were mostly not significant due to the low number of samples (n=5) and the standard deviation partly being very high.

MANDELL et al. (1997) have carried out experiments with fishmeal supplementation to produce meat products with high concentrations of ω-3 fatty acids. This procedure is currently not commonly used in finishing diets due to its high cost and because protein requirements decrease during late phases of growth. In this trial Charolais steers (final body weight 550-590 kg) were fed maize and alfalfa silage plus 0, 5 or 10 % herring meal. Fishmeal feeding increased concentrations of palmitic acid (27.5-28.9 %) and ω-3 fatty acids (but not total PUFA with ca. 5.3 %), including eicosapentaenoic (0.1-0.7 %) and docosahexaenoic acid (0.04-0.28 %), and decreased concentrations of stearic acid (13.6-11.9 %) and arachidonic acid (0.7-0.3 %) in MLD, which had an intramuscular fat content of ca. 5 % that was not influenced by fish meal supplementation. Oleic acid was determined to be ca. 44 %, myristic, palmitoleic and linoleic acid to be ca. 3.5 %, and the long-chain and PUFA (linolenic, γ-linolenic, eicosatrienoic, docosatetraenoic and docosapentaenoic acid) followed with each up to 0.4 %. The SFA content with ca. 45 % and its contributing fatty acids are comparable to the present Young Bulls trial, but the MUFA content of ca. 49 % is about 10 % higher, which is the missing 10 % in the SFA content. It was expected that this study would provide higher levels of PUFA, since ruminal microorganisms do not hydrogenate C20 and C22 ω-3 fatty acids to any significant extent. But the fish meal seems only to elevate long-chain ω-3 fatty acids, whereas especially linoleic acid and arachidonic acid remain rather low or even decrease. In non-ruminants, fish oil supplementation also decreased arachidonic acid in poultry and swine. The ω-3 fatty acids found in fish meal or fish oil interfere with synthesis of arachidonic acid from linoleic acid or ω-3 fatty acids may be competing with arachidonic acid for deposition sites in phospholipids (MORGAN et al., 1992). Anyway, consumption of 110 g beef from this trial would provide maximum 10 % of the recommended intakes of ω-3 fatty acids.

Such as LARICK et al. (1989) have demonstrated the influence of genetics on the neutral lipid fatty acid composition of Bison, Hereford, and Brahman steers, different studies were designed by BOYLSTON et al. (1995), XIE et al. (1996) and ZEMBAYASHI & NISHIMURA (1996) to further examine the influence of breed towards fatty acid pattern. The latter examined the genetic and nutritional influences on fatty acid composition of subcutaneous and intramuscular neutral lipids and intramuscular phospholipids of steers from two dam breed-types (Japanese Black and Holstein), fed concentrate (maize, barley, wheat) plus rice straw on two different nutritional planes and slaughtered between 480-630 kg. Fatty acids were not effected by dam breed-type, but differed among sires and also in dependence of nutritional plane. According to mean values given, intramuscular neutral lipids contained oleic acid with 50.1 % as major fatty acid, followed by 23.9 % palmitic acid, 10.1 % stearic acid and 4.3 % palmitoleic acid. The minor fatty acids myristic, linoleic, eicosenoic, margaric, heptadecenoic, myristoleic and linolenic acid were present with 2.6 % down to 0.8 %. In this case, SFA (38.2 %) and PUFA (3.0 %) were lower

than in our present trial, whereas the content of MUFA was extremely high with 58.8 % - comparable to the study by HECKER et al. (1975).

BOYLSTON et al. (1995) presented a MUFA content even higher than this. Beef from Japanese Wagyu has significantly higher ratios of MUFA to SFA than other beef breeds. But Japanese Wagyu are also noted for their ability to marble without depositing excessive external fat which contributes to the characteristic taste and tenderness of the beef (XIE et al., 1996). BOYLSTON et al. (1995) compared MLD from Japanese Wagyu, American Wagyu, Longhorn, Angus and U.S. Choice, which were fed the same typical Japanese feeding practice for 524 days, consisting of wheat straw, barley, alfalfa and maize. MLD was boiled or roasted prior to analysis. The neutral lipid contents of the five breeds ranged from 9.5 % in U.S. Choice to almost 24 % in American and Japanese Wagyu, which is by far higher compared to the present trial. Among the experimental animals, Japanese Wagyu had the highest content of MUFA (68.3 %; thereof 58.2 % oleic acid and 5.3 % palmitoleic acid), but only 1.5 % PUFA in the neutral lipid fraction. Japanese Wagyu beef instead had lower percentages of palmitic (23.3 %) and stearic acid (7.0 %) than the other beef sources, resulting in a very low SFA content of only 31.9 %. Out of the five different breeds, Angus had the highest SFA content with 42.5 % and the lowest MUFA content (56.4 %), but still none of the results from this study are comparable with the present Young Bulls trial. The increased oleic acid concentration in fatter animals could be the result of elevated microsomal desaturase activity with increased animal age or increased absorption of dietary fatty acids resulting from decreased ruminal biohydrogenation with grain feeding. The bacterial biohydrogenation in the rumen could be limited by the reduced ruminal pH caused by high-concentrate diets and, therefore, more unsaturated fatty acids enter the small intestine for absorption and incorporation into tissues, leading to the increase of MUFA with increased duration. In contrast, the elevated content of MUFA in adipose tissue of the Wagyu breed could be more likely caused by elevated stearoyl-CoA desaturase activity within adipose tissue, which converts SFA to their ω-9 MUFA counterparts (BOYLSTON et al., 1995; XIE et al., 1996). The low PUFA content may be due to the different analytical procedure again, as BOYLSTON et al. (1995) determined only triglycerides. On the other hand, long-chain and PUFA may have been lost during the heating process, as fatty acids can react to carbonyl compounds upon heating (IGENE & PEARSON, 1979; LARICK et al., 1989). Another aspect, which was already mentioned, is the significant variability in the lipid contents between the trials, because the fatty acid pattern changed to be more like the ones from adipose tissues with increasing fat content.

According to ALLEN & FOEGEDING (1981) muscle lipids vary in quantity and composition within the variety of avian, aquatic and mammalian muscle foods. Additionally, because of the confounding of age, plane of nutrition, fatness and other extrinsic factors, it is difficult to compare the fatty acid composition of different breeds. Furthermore, the content and composition of muscle lipid differ within an animal depending upon the muscle function, and some changes in fatty acid composition can be brought about by dietary fat modification. On the other hand, BLUNK et al. (1992) found the influence of feed intensity on the fatty acid pattern in cattle not to be significant, as well as ALLEN & FOEGEDING (1981) and GURR (1997) stated that it is difficult to increase the PUFA content of ruminant meats because of the extensive degree of biohydrogenation of dietary fatty acids by rumen micro-organisms.

As fatty acid profiles of ruminants are difficult to modify via dietary manipulation, XIE et al. (1996) decided that a modification of fatty acids by selective breeding might be more effective. Their focus was on the total intake of SFA and MUFA:SFA ratios, respectively, so they chose crossbred Japanese Wagyu- and Angus-sired steers to feed on basis of barley and alfalfa. Wagyu-sired steers had higher marbling, maturity and quality scores, larger MLD areas and lower fat thickness (among others). For MLD (and the same counts for subcutaneous fat), crossbred Wagyu-sired steers had slightly higher percentages of palmitic (28.5/27.2 %), palmitoleic (4.1/3.4 %) and myristic acid (2.9/2.7 %), and slightly lower percentages of stearic acid (12.2/13.8 %) than Angus-sired steers, as well as lower contents of linoleic acid (2.8/3.2 %) and PUFA (3.3/3.9 %). Not influenced by breed were oleic acid (ca. 47 %), SFA (43.5 %) and MUFA (ca. 52 %), as well as the ratio MUFA:SFA (1.19/1.21). Again, these concentrations are not quite comparable with the ones from the present trial. Explanations are again the proposed factors (breed, sex, nutrition, fatness, etc.), that have been described in detail before. Concerning the MUFA:SFA ratio, the Young Simmental Bulls from the present trial show only a value of 0.9, but the experimental animals of BOYLSTON et al. (1995) varied from 1.3 for Angus steers to 2.1 for Japanese Wagyu steers.

A favourable balance is also desired between PUFA and SFA as well as between ω-6 and ω-3 fatty acids. Recommendations in the UK are to reduce the intake of short- and medium chain SFA to a PUFA:SFA ratio of 0.45 and the intake of ω-6 polyunsaturates relative to ω-3 PUFA to below 4.0. Therefore, in ruminants, the challenge of dietary manipulations is to increase the PUFA:SFA ratio whilst retaining the values for ω-6:ω-3. Concerning the values for our bulls, both recommendations are not met. The problem for the PUFA:SFA ratio is the low concentration of PUFA because of the hydrogenating action of the rumen bacteria that convert a high portion of PUFA into fatty acids with fewer double bonds. But this saturating effect of the rumen can be overcome by feeding PUFA which are protected either chemically, by processing or naturally, e.g. within the seed coat or the intact organelle (e.g. chloroplasts in grasses). And already 20 years ago, it was possible to produce meat products and milk rich in PUFA by feeding “protected” (encapsulated) oilseed supplements (ALLEN & FOEGEDING, 1981). On the other hand, it appears that at least the long-chain C20 and C22 ω-3 PUFA from fish oils avoid rumen hydrogenation to a large extent and are absorbed intact in the small intestine (WOOD & ENSER, 1997; MANDELL et al., 1997).

Unfortunately no data appear to be available for a comparison of MST, liver or kidney fat. MST seems to be the easiest one to discuss, as it can be expected from the similarity of the fatty acid patterns between MLD and MST in this present trial, that the argumentation done for MLD can be applied to MST as well. Kidney fat was expected to be similar to subcutaneous fat, but from data published it seems that subcutaneous fat is more comparable to MLD rather than to kidney fat from the present trial. But that may as well be due to the strong differences that occurred anyway between this present trial and the others published. The closest was RULE et al. (1997), who found high contents of SFA (up to 53 %), but the relation between palmitic, stearic and oleic acid was different. Additionally, those animals were rather young, so that this would be a poor comparison. No data could be found in literature to compare liver values, so that the only ones available are from the subsequent trial.

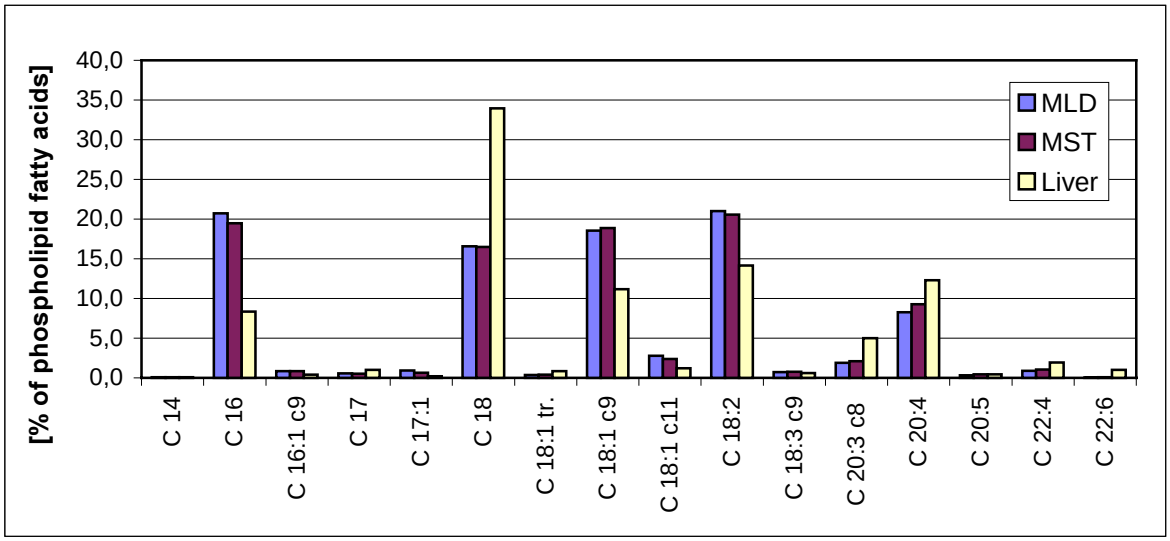

Figure 9: Young bulls "Supplementation" – selected fatty acids of phospholipids in different tissues

According to expectations the fatty acid distribution in the phospholipid fractions of muscle tissues looked different compared to total fat (see fig. 9, also refer to appendix 8.7.2), which is confirmed by ZEMBAYASHI & NISHIMURA (1996). In MLD and MST 16-21 % for each palmitic, stearic, oleic and linoleic acid were determined as well as almost 10 % arachidonic acid. Vaccenic and eicosatrienoic acid were found to be about 2-3 % and palmitoleic, heptadecenoic, linolenic and docosatetraenoic acid to almost 1 %. The value for oleic acid was ca. 15 % lower than in total fat, in favour of stearic and arachidonic acid which yielded now 10 and 5 % more, respectively, and increased the PUFA content to ca. 34 %. The SFA content was about 5 % and the MUFA more than 15 % lower than in total fat of muscle tissues, mainly due to palmitic and oleic acid. In liver tissue patterns of total fat and phospholipid fraction were alike, containing ca. 10 % palmitic, oleic and arachidonic acid, but more than 30 % stearic acid. Most other detectable fatty acids were below 1 %, except for eicosatrienoic acid (5 %), docosatetraenoic acid (2 %) and docosahexaenoic acid (1 %).

Unlike the neutral lipids, phospholipids and cholesterol are membrane-associated and the phospholipid portions of muscle lipids are essential to muscle because of their role in the structure and function of the muscle cell and its organelles. Phospholipids contain a limited range of fatty acids in which PUFA of the ω-6 family predominate. Because biological membranes operate optimally within a limited range of fluidity, and because fluidity is in part influenced by fatty acid composition, the latter is conservative and not extensively influenced by diet or other environmental factors. Some changes in fatty acid composition, though, can be brought about by (very substantial) dietary fat modification (ALLEN & FOEGEDING, 1981).
Polar lipids account for a minor fraction of lipid in beef, with contents ranging from 0.39-1.6 % in beef (ca. 7.5-14 % of total fat) and decreasing as the concentration of total lipid in beef muscle increases (CAMPBELL & HARRILL, 1971; LARICK et al., 1989; BLUNK et al., 1992; BOYLSTON et al., 1995). Therefore inclusion of marbling fat with muscle when sampling for analysis will tend to give a misleading view of the structural fat composition, diluting the proportion of the PUFA (GURR, 1997).

BLUNK et al. (1992) analysed phospholipid fatty acid patterns in MLD of bulls, steers and heifers of German Fleckvieh (= Simmental), fed with maize silage and concentrate up to 650 kg. Fatty acids were found to be equally distributed among classes of SFA, MUFA and PUFA. The major fatty acids were oleic (29 %), palmitic (19 %), linoleic (15 %), stearic and arachidonic acid (13 %). Other fatty acids above the detection limit were palmitoleic, heptadecanoic, vaccenic, eicosatrienoic and docosatetraenoic acid with about 1.6-2.9 % as well as linolenic and eicosapentaenoic acid with 0.6-0.7 %. These data were not completely concurrent with the results from this present trial, as the results for oleic acid differed for about 10 %, as well as linoleic, stearic and arachidonic acid differed for about 3-5 %. Therefore the MUFA content was apparently higher than in the present trial, but the PUFA contents were comparable, just differing in the relation of linoleic to arachidonic acid. It was expected that results would be closer, as animal breed, age and feeding were highly comparable to the present trial. So it may be due to the influence of sex and additional extrinsic factors that were not as similar as expected.

ITOH et al. (1999) had also determined the polar muscle fraction for the heavy weight Simmental steers that were raised with maize grain and 30 % pasture silage, but although their results were comparable to the present study concerning the neutral fraction, the results were very different in the polar fraction. Main fatty acid was oleic acid (31 %), followed by 14 % palmitic and stearic acid each and 12 % linoleic acid, while those four fatty acids were all about 17-21 % in the present study. There were also differences in the minor fatty acids, e.g. the arachidonic acid content was only about half our value, while eicosa- and docosapentaenoic acid were much higher. Consequently also SFA (28 %), MUFA (36 %) and PUFA (25 %) were not comparable to the present study. As the breed was the same, the differences should also be explained by the other factors, such as the different sex, the high intramuscular fat content, feed, etc.

BOYLSTON et al. (1995) found that the fatty acid content of the polar fraction of the five different breeds they analysed was not as variable as was the neutral lipid fraction. But still, contents of 19.6-38.8 % oleic acid, 16.2-18.6 % palmitic acid, 12.9-22.2 % linoleic acid, 6.9-8.0 % stearic acid, 4.9-7.1 % arachidonic acid, 2.0-2.8 % palmitoleic acid, 1.7-3.6 % vaccenic acid and up to 0.4 % linolenic acid were determined in MLD of those steer types. These values also do not fit to the ones determined in the present trial, as none of the chosen breeds e.g. reached a stearic acid content of 17 % and also strong variations in oleic acid and linoleic acid occurred. According to this, even the maximum SFA and PUFA contents of ca. 30 % and 27% were not high enough compared to the results from the present trial. And the low oleic acid and MUFA contents were only reached by U.S. Choice steers. Reason for these divergences besides breed, nutrition and sex in this case may be the heating process, which could have changed the fatty acid pattern.

ZEMBAYASHI & NISHIMURA (1996) were closest to the present study concerning the contents of SFA (40.6 %), MUFA (25.5 %) and PUFA (33.9). But the single fatty acids did not match as good. In intramuscular phospholipids of two different dam-breed steers contents of palmitic (12.9 %), stearic (8.8 %) and especially arachidonic acid (0.06 %) were about 8 % lower. Instead, eicosatrienoic acid (9.7 %) and margaric acid (5.1 %) were extremely high. Oleic acid (20.0 %) and linoleic acid (20.7 %) as well as the other fatty acids were about in the range of the values determined for this present trial, which is especially surprising for oleic acid, which

usually has had the strongest deviation. It could be seen from almost all studies that values for fatty acids can vary quite much and probably the strongest influence is by breed and nutrition. Such as LARICK et al. (1989) attributed the observed genetic differences in fatty acid profiles (of Bison, Hereford, and Brahman steers fed the same diets) to the incorporation of different amounts of endogenously produced fatty acids.

Also for phospholipids no data could be found to compare liver values, except the ones from the subsequent trials. Kidney fat was not analysed for fatty acid pattern of phospholipids, as the concentration in total fat is very low. For the same reason also ZEMBAYASHI & NISHIMURA (1996) categorised lipids extracted from subcutaneous samples as neutral lipids.

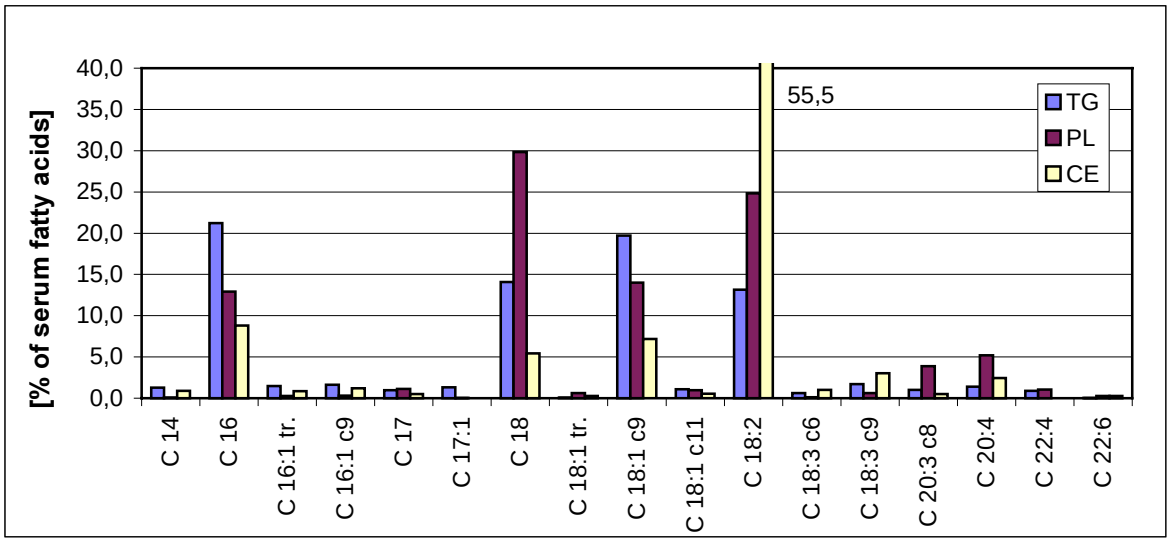

Figure 10: Young bulls "Supplementation" – selected fatty acids of serum fractions
(TG = triglycerides, PL = phospholipids, CE = cholesterol esters; n = 4)

Serum fractions also differed greatly from each other (see fig. 10, also refer to appendix 8.7.2): The triglyceride fraction consisted mainly of palmitic and oleic acid (ca. 20 %) as well as stearic and linoleic acid (13-14 %). Within the 1-2 % range were following myristic, palmitelaidic, palmitoleic, margaric, heptadecenoic, vaccenic, linolenic, eicosatrienoic and arachidonic acid. With contents of 38 % SFA, 26 % MUFA and 19 % PUFA the pattern was in-between total fat and phospholipid composition of MLD. The phospholipid fraction of serum contained besides a rather high amount of 30 % stearic acid also 25 % linoleic and 13 % palmitic acid. Compared to the other fractions, striking high contents of eicosatrienoic and arachidonic acid (3-4 %) were determined as well as the long-chain PUFA docosatetraenoic and docosahexaenoic acid (1.0 and 0.3 %, respectively). This resulted in 44 % SFA, only 16 % MUFA, but 36 % PUFA; almost like the phospholipid pattern in liver tissue. The cholesterol ester fraction however, was characterised by an even higher content of 70 % PUFA (caused by 62 % linoleic acid) and therefore not comparable to any tissue analysed before. Other fatty acids present were palmitic acid (8.8 %), oleic acid (7.2 %), stearic acid (5.4 %), linolenic acid (3.0 %), arachidonic acid (2.4 %) and palmitoleic acid (1.2 %) and further minor fatty acids were detected below 1 %. Due to the high PUFA content, SFA and MUFA were extremely low with 16 and 11 %, respectively.

Except for the publication by HECKER et al. (1975) no other data about fatty acid profile of serum from cattle appear to be available. Even in this publication fatty acid composition was given only for serum itself but not for the different lipid classes. The main fatty acids described were palmitic, stearic, oleic and linoleic acid with each about 20-25 %, accompanied by 1-3 % of each myristic, myristoleic, pentadecanoic, palmitoleic and margaric acid. Long-chain and PUFA were not listed. It is difficult to compare both results, but according to these values the pattern could be a mixture of those from triglycerides and phospholipids. In that case the cholesterol esters seem to be not represented to a high extent or they must have had a less extreme fatty acid composition. This divergence of results could be due to the fact that HECKER´s study was performed a long time ago and therefore either breed or feeding have changed or the analytical method was not accurate enough.

Fatty acid patterns for human plasma, split up into the different lipid classes and obtained with the same analytical method, have been determined by FRITSCHE et al. (1998). In that study the fraction of cholesterol esters also yielded 65 % PUFA, due to 53 % linoleic acid, together with 13 % SFA and 23 % MUFA. This result was almost equivalent to the results from this trial. Additionally, the values for SFA (44 %), MUFA (17 %) and PUFA (40 %) in the phospholipid fraction were concurrent with those obtained in the present trial. The triglyceride fraction differed though, mainly due to a high value of MUFA (47 %) and a lower SFA content (33 %).

From the results of this present trial it seems that patterns were not significantly affected by α-tocopherol supplementation, so that it may be concluded that α-tocopherol is not favouring the incorporation of PUFA. This conclusion was confirmed by SCHAEFER et al. (1995), who found no differences in ether-extractable lipid, fatty acid profile or cholesterol of MLD or *gluteus medius* due to supplementation. Also CHAN et al. (1996) could not detect effects of dietary α-tocopherol supplementation to fatty acid composition when measuring muscle microsomes.
A similar result was reported in pork by MONAHAN et al. (1992), who proposed that α-tocopherol supplementation did not influence the deposition of fatty acids in the muscle or adipose tissue of pigs fed either beef tallow or soya oil diets. In chicken different oils significantly affected the fatty acid composition of the neutral lipids and, to a lesser extent, the fatty acid composition of the phospholipids. However, α-tocopherol supplementation had no effect on the fatty acid composition of neutral and polar lipids (LIN et al., 1989). And the same effect was noticed by SCHWARZ (1996) for carp: Dietary fatty acid composition affected fatty aid patterns of tissues of carp significantly, but this was correlated with the fat content of the diet. Varying α-tocopherol content results in no or only very slight variations in the fatty acid pattern of the tissues.
In the feeding experiment performed by FLACHOWSKY et al. (1994), Schwarzbunte bulls receiving rapeseed supplementation showed significantly enhanced α-tocopherol concentrations in all body samples. But differences in fatty acid patterns were mostly not varying significantly. In comparison with the fatty acid pattern of rapeseed and with modifications of fatty acid pattern after feeding plant oils to non-ruminant species, the changes for cattle were rather small. Main reason is probably the hydrogenation of unsaturated fatty acids in rumen, as mentioned before. But anyway the small changes could have been resulting from either α-tocopherol supplementation or, with a higher probability, from supplementation of rapeseed fatty acids.

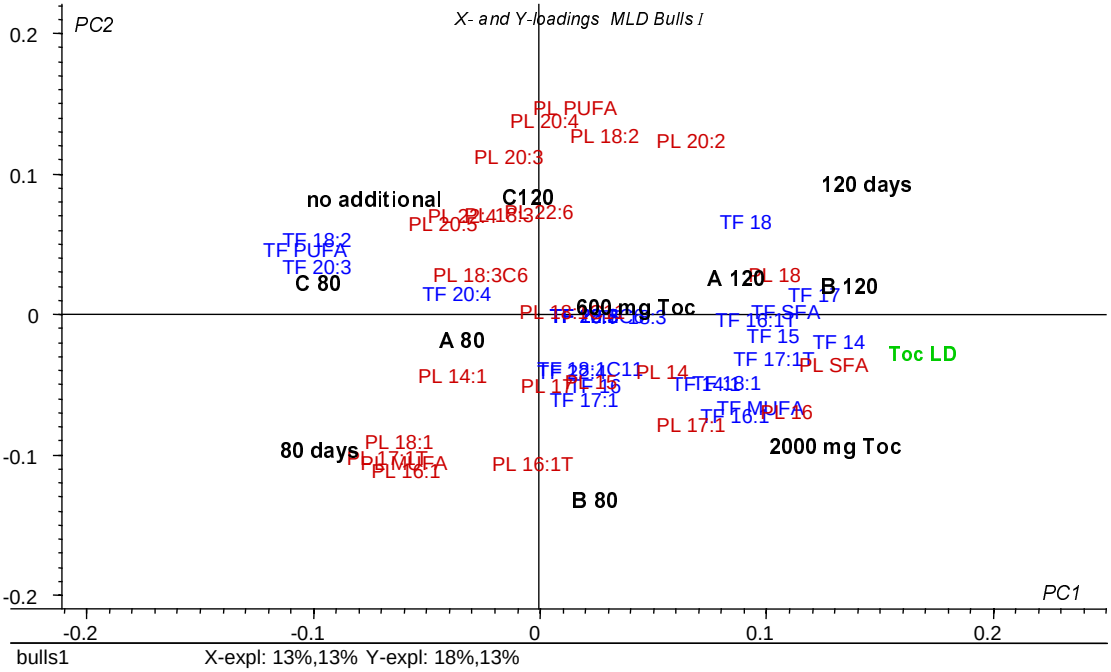

Figure 11: Young bulls "Supplementation" – PLSR2-plot for MLD
(C = control group, A = group fed 600 mg α-tocopheryl acetate, B = group fed 2000 mg α-tocopheryl acetate, 80 - 120 referring to duration of supplementation before slaughter, TF = total fat, PL = phospholipid fraction)

Data from this present trial were therefore subjected to Multivariate Analysis, as to check if actually no influence could be detected. And surprisingly, the resulting loadings plot (see fig. 11, also refer to the scores plot - appendix 8.7.3) showed a certain probability that fatty acid patterns were affected by feeding. The design variables of the animal groups are moving from the second to the fourth quadrant with increasing α-tocopherol supplementation, while the duration of feeding influences them to shift into the directions of the first and third quadrant. The contents of PUFA in phospholipids and especially total fat seemed to be closest related to the control groups, although it was expected that exactly the opposite would happen, as these fatty acids should be best protected at higher concentrations of α-tocopherol. SFA and MUFA instead tended to be higher with increasing α-tocopherol contents, the latter especially with shorter duration and the SFA with longer duration of supplementation. Similar correlations were also determined for the other tissues, but the percentage of explanation was too low for any definite statement whether α-tocopherol supplementation influenced fatty acid composition or not. It definitely did not have such an overwhelming effect, which would make meat industry or consumers requesting a change of feeding strategy towards high supplementation with α-tocopherol.

4.2 Experiment II: Long-term α-Tocopherol Supplementation for Young Bulls

As could be seen from the previous trial, a difference between the short-time feeding strategies of 80 and 120 days could only be determined for those groups that received the high dosage of 2000 mg α-tocopheryl acetate/kg. However, the recommended level of 3-4 mg/kg MLD was not even obtained for the group that received these 2000 mg/kg for 120 days. Assuming that the period of 120 days was still too short for α-tocopherol equilibration and that a plateau was not reached within these four months, a long-time feeding experiment, such as they were also performed by several US-researchers, should give further indications about rate and extent of α-tocopherol uptake into tissues. Therefore, the objective of this second study was to verify the earlier experiment by evaluating the effect of long-time feeding (using a conventional middle European system) of a moderate level of α-tocopherol supplementation on α-tocopherol contents of tissues, resulting fatty acid patterns as well as carcass and meat quality of finishing bulls.

4.2.1 Experimental Design

Twenty-four bulls (German Fleckvieh = Simmental; 11-12 per treatment, see tab. 6) were supplemented with 1000 mg α-tocopheryl acetate per animal per day for either 180 days or 270 days prior to slaughter. The bulls in the control group received the recommended daily allowance of α–tocopherol. The animals had initial life-weights of 307 kg (270 days to slaughter) and 440 kg (180 days to slaughter) and at slaughtering the final overall mean life-weight was 650 kg.

Table 6: Young bulls "Long-term", divided into feeding groups (n = number of animals)
(C = control group, D 180 = group fed 1000 mg α-tocopheryl acetate for 180 days, D 270 = group fed 1000 mg α-tocopheryl acetate for 270 days before slaughter, n = number of animals)

	group C	Group D 180	Group D 270
supplementation with α-tocopheryl acetate	without additional α-tocopherol	1000 mg/day, 180 days	1000 mg/day, 270 days
number of animals	n = 12	n = 11	n = 12

Feed and feeding procedure were the same as described before (see 4.1.1)

4.2.2 Slaughter and Sampling Procedures

Procedure and samples taken were the same as described before (see 4.1.2). For measurements of α–tocopherol contents and determination of fatty acid patterns blood samples were taken as well as samples from the liver, kidney fat, the foreloin (*Musculus longissimus dorsi* - MLD) and the round (*Musculus semitendinosus* - MST).
α-Tocopherol contents were determined once for every single animal. For fatty acid pattern only six samples per group were chosen randomly and their tissues were analysed once.

4.2.3 Results from Co-operating Institutes

The different levels of α-tocopherol in the diet did not influence energy intake, life-weight gain (ca. 1290 g/day) and carcass quality. The intra-muscular fat content of the MLD was twice as high as in the last trial, with an average of 3.0 %, while MST again contained 1.1 % fat, corresponding to the normal values for young beef bulls. The supranutritional dose of α-tocopherol had positive effects on the stability of the red colour and delayed fat oxidation, measured as TBARS numbers, as in the last trial. While in the first trial the longer duration (120 days in comparison to 80 days) had a stronger positive effect than the amount of α-tocopherol, the prolonged supplementation up to 180 and 270 days in this experiment did not further improve the positive influence on oxidative stability. (SCHWARZ 1998, personal communication).

4.2.4 Results and Discussion of α-Tocopherol Contents

In this second study the supplementation of α-tocopherol resulted in a highly significant increase of tissue concentrations compared to the control group. Extension of the supplementation period from 180 to 270 days also showed a highly significant effect throughout all tissues (see fig. 12; also refer to appendix 8.7.1). Again tissues differed from each other: liver (1.0-8.1 mg/kg) and kidney fat (1.3-7.2 mg/kg) accumulated more α-tocopherol than did MLD (0.4-2.9 mg/kg), MST (0.3-2.6 mg/kg) and serum (0.4-2.3 mg/kg) and the accumulation appears to occur in a dose- and duration dependent manner.

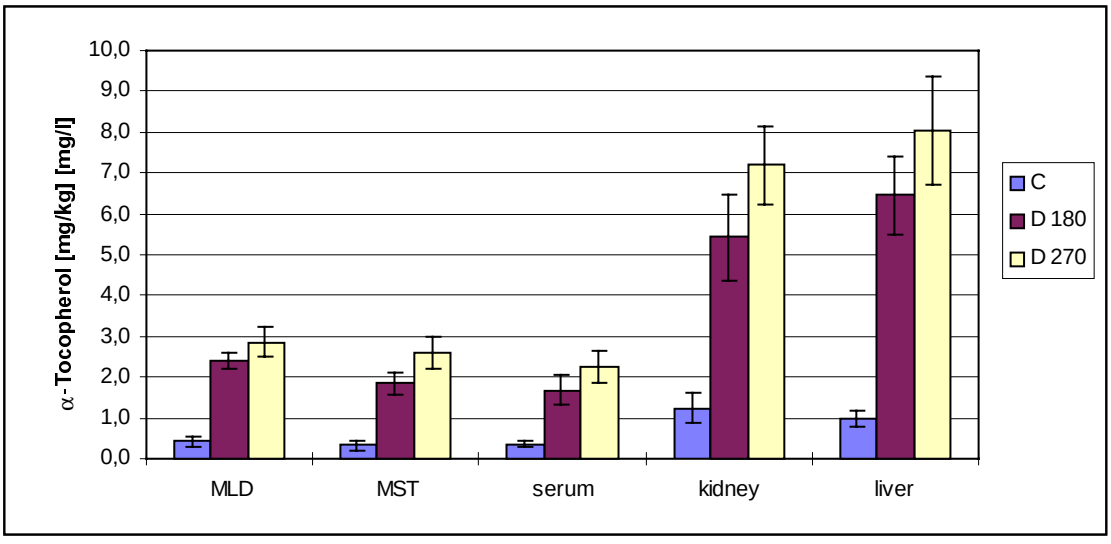

Figure 12: Young bulls "Long-term" - α-tocopherol contents in different tissues depending on feeding
(C = control group, D 180 = group fed 1000 mg α-tocopheryl acetate for 180 days, D 270 = group fed 1000 mg α-tocopheryl acetate for 270 days before slaughter, n = 11-12)

The MLD of control group C (see fig. 12, also refer to appendix 8.7.1) contained only ca. 0.4 mg α-tocopherol/kg. That corresponds with the value determined in the previous trial. The supplementation with 1000 mg α-tocopheryl acetate was increasing the content to 2.4 mg/kg when fed for 180 days and 2.9 mg/kg when fed for 270 days (group D). Assuming, that feeding 1000 mg/kg for 180 days leads to about the same absolute uptake as 2000 mg/kg for 90 days, group D 180 should show a similar α-tocopherol content as group B 80. This was not the case as they contained 1.5 and 2.4 mg/kg, respectively. On the other hand, groups D 270 and B 120 should differ more in α-tocopherol concentration as they actually did. 2.8 mg/kg compared to 2.9 mg/kg may suggest that there is a certain limit of α-tocopherol uptake into MLD, especially when considering that within the three months between D 180 and D 270 the increase was only 0.5 mg/kg, whereas the six months before it was 2.0 mg/kg. It is confirmed also by PARKER (1989), ARNOLD et al. (1993a) and ROSENBAUER et al. (1996) that α-tocopherol uptake in all tissues, except for liver and adipose tissue, will reach a certain plateau. α-Tocopherol accumulation in *lumborum* muscle e.g. equilibrated with dietary α-tocopherol intake after 12-18 weeks. Additionally, α-tocopherol content of muscle biopsies from *semimembranosus* muscle of beef steers indicated that by day 56 approximately 90 % of the α-tocopherol that would be present at slaughter had been accumulated. This indicates that perhaps a lengthy feeding period is not needed to optimise α-tocopherol accumulation (GARBER et al., 1996).

Concerning the α-tocopherol contents in MLD, there are no comparable experiments published, especially not for bulls. The few experiments with a supplementation of 1000 mg/kg were performed for only 125-145 days, and just *semimembranosus* and *gluteus medius* were analysed. Those muscles contained 3.8 and 6.1 mg/kg in crossbred steers and 2.3 and 5.2 mg/kg in Holstein steers, respectively (GARBER et al., 1996) and were therefore higher, even with a shorter feeding period. Also higher in α-tocopherol content were Holstein and crossbred steers from long-time feeding experiments by ARNOLD et al. (1993b), who used only 500 mg/kg for 252 days, but yielded concentrations of 4.1-4.2 mg/kg in *lumborum* muscle and 5.0-5.5 mg/kg in *gluteus medius*. Expectedly higher were results by MITSUMOTO et al. (1993), who fed Holstein steers with 1500 mg/kg for 232 and 252 days, which resulted in *lumborum* contents of 6.1 and 6.8 mg/kg, respectively.

Contents for MST and serum were even lower than MLD throughout all feeding groups (in contrary to the previous Young Bulls 1 and the following steers' experiments). The basic contents of the control group (0.3 mg/kg and 0.4 mg/l, respectively) were increasing for each increment of α-tocopherol supplementation to 1.9 mg/kg and 1.7 mg/l (D 180) and reaching 2.6 mg/kg and 2.3 mg/l (D 270), respectively. These values were also lower than those from the previous trial were: in MST already 1.9 mg/kg was reached for group A 120 and 2.9 mg/kg were determined for group B 80. And for serum the maximum of this trial was less than group B 80 (3.1 mg/l) of the last trial.

In literature again it was difficult to find comparable results. There were no further information for MST so that it is necessary to refer to the same results as mentioned already before in the paragraph about MLD, which means that relatively seen the MST values were even lower than already the MLD contents were. For serum there are some concentrations published, but again

none of the experiments were performed under the same conditions. GARBER et al. (1996) found already 4.9 and 3.0 mg/kg for crossbred and Holstein steers supplemented with 1000 mg/kg for only 125 and 145 days, respectively. In the long-time studies by ARNOLD et al. (1993b) contents of 1.4-1.8 mg/l for control group and 4.4-5.6 mg/l for steers receiving only 500 mg/kg for 252 days were determined. These results are already significantly higher and can only be explained by differences in feed, breed, sex and / or other environmental circumstances, as it was described in detail in the chapter of the first Young Bulls experiment.

In liver tissue again the highest levels occurred. Contents of 1.0 mg/kg (control group C), 6.5 mg/kg (D 180) and 8.1 mg/kg (D 270) were determined. These values showed the strongest divergence towards the results from the previous trial, as they were significantly lower than in Young Bulls experiment I. The maximum level of 8.1 mg/kg corresponds to a level between group A 120 and B 80, comparable to serum, and is only half of the content measured for group B 120. Values described in literature are significantly higher, such as GARBER et al. (1996) found 15-16 mg/kg in steers supplemented with 1000 mg/kg for only 125-145 days and ARNOLD et al. (1993b) still determined 10-13 mg/kg in steers supplemented with only 500 mg/kg for 252 days.
In contrary, the results for kidney fat were in confirmation of the previous study, just slightly lower. Starting with a content of 1.3 mg/kg in the control group, the level increased to 5.4 mg/kg (D 180) and 7.2 mg/kg (D 270). In this case there are no values given in the literature for a comparison.

The rate and extent of α-tocopherol equilibration could be a possible explanation when comparing the results of the second with the first trial. The α-tocopherol contents in serum and other tissues mirror the dose and duration of α-tocopherol given. Increases in MLD but not plasma are consistent with the conclusion of ARNOLD et al. (1993a) that the skeletal muscle α-tocopherol pool equilibrates with dietary α-tocopherol intake more slowly than does the plasma pool (LIU et al., 1996b). In this present study it was shown very clearly that serum and liver had lower concentrations than in the previous trial, whereas MLD and kidney fat reached about the same levels. Especially from the liver value of group D 270 compared to B 120 can be seen that this time only half the α-tocopherol was added. So, liver is not a final storage deposit, as it is still involved in metabolic processes that inhibit an accumulation. This is also confirmed by JENSEN et al. (1988) and SCHAEFER et al. (1995), who stated that equilibration between tissue concentration and dietary intake was more rapid for blood and liver as well as other inner organs than for MLD. This led to the conclusion that the concentrations of α-tocopherol in serum and liver reflect the immediate nutritional status of the animal, whereas the α-tocopherol concentrations in adipose and skeletal muscle tissues reflect its long-term nutritional history. In this trial therefore the suggested order of tissue concentrations for long-term supplementation was yielded: inner organs (liver, heart, lung) > adipose tissues > muscle tissues > blood (MONAHAN et al., 1990b; SHEEHY et al., 1991).

4.2.5 Results and Discussion of Fatty Acid Patterns

The results in this second experiment (refer to appendix 8.7.2 and 8.7.3 for scores plot) were not very different from those in the first experiment. The deviation between animals within a group was about the same as between groups; therefore only mean values are listed in the appendix.

Oleic acid was again the major fatty acid (37 %) in total fat of the muscle tissues MLD and MST, followed by palmitic acid (25 %), stearic acid (15 %), linoleic acid (6 %) and palmitoleic acid (3 %). The main differences therefore were the MUFA content to be about 5 % higher than in the first trial, due to elevated contents of oleic acid, and the PUFA content to be about 5 % lower, due to the content of linoleic acid. Reasons for these differences were not obvious. As breed, sex, basic feeding, slaughter weight and most of the other extrinsic factors were the same as in the first trial, it could be just guessed that maybe the procedure of sample handling and/or analysis was slightly different and could have caused the low differences. Samples from this study had been stored in frozen condition for a longer time, so that changes in cellular structures might have occurred. In that case e.g. the PUFA might have been attacked and broken down, which would explain the lower values. Additionally the conditions of the GC analysis had been different between the two trials, but that should not lead to such differences. In both cases however, the same tendency should show up in all tissues and in all fractions. A third option might be the influence of long-term supplementation with α-tocopherol, which would not necessarily affect all tissues or fractions, caused by their different physiological characteristics.

Liver tissue contained only about 12 % oleic acid and 8 % palmitic acid in the total fat, but more than 30 % stearic acid and 12 % of each linoleic and arachidonic acid. These results were therefore confirming those from the first trial, as there is only a shift of maximum 1 % in some fatty acids.

Kidney fat contained less than 2 % PUFA, but ca. 25 - 30 % of each palmitic acid, stearic acid and oleic acid. The content of stearic acid and linoleic acid were slightly lower than in the first trial and resulted also in lower contents of SFA and PUFA. The content of MUFA is slightly higher, partly due to elaidic acid.

Concerning the values for MLD, a comparison with the literature this time yielded better results. As in all of the publications especially the values for the PUFA content were too low and the ones for MUFA too high, compared to the results from the previous trial, now some values fit better. RULE et al. (1997) had studied the effect of duration towards fatty acid pattern of crossbred steers and found almost the same composition as the present trial, especially for the younger animals. Also FLACHOWSKY et al. (1994) found similar values for the bulls without rapeseed supplementation, with just a slightly lower content of MUFA. As this was also a European experimental design with bulls, such similarities were expected rather than for comparison with US trials. MANDELL et al. (1997) on the other hand determined lower contents of PUFA (5.3 %) but higher contents of MUFA (49 %) as a consequence of fish meal feeding to steers. Even higher were MUFA contents in the feeding experiments with different breeds.

ZEMBAYASHI & NISHIMURA (1996) as well as XIE (1996) determined already 50 % oleic acid, whereas BOYLSTON et al. (1995) measured 56-58 %, resulting in MUFA contents of almost 60-70 %. These breeds were obviously not comparable with the one from this trial and additionally feeding and sex as well as probably most other extrinsic factors were different.
A comparison of the results for MST, liver and kidney tissue with publications of other authors cannot be performed, due to the reasons already described in the previous chapter.

The fatty acid distributions in the phospholipid fractions of the muscles MLD and MST were determined to be quite different from the results of the previous study. These tissues contained higher concentrations of oleic acid (25 % instead of 18 %), but lower concentrations of palmitic and linoleic acid (18 % instead of 20-21 %), as well as stearic acid (14 % instead of 16 %). The content of ca. 9 % arachidonic acid was confirmed. As a consequence, the MUFA content increased with ca. 6 % up to 30 %, whereas the concentration of SFA dropped about 4 % to 33 % and the PUFA content remained almost stable. Due to this high divergence, an explanation is as difficult as for the total fats, especially as it is stated in literature (ALLEN & FOEGEDING, 1981) that phospholipid fatty acids cannot be extensively influenced because of their biological functions. More recent research though did show that e.g. increasing levels of intramuscular fat were associated with changes of fatty acid patterns, such as variability in fatty acid composition may also arise from feed type or age of animal (ITOH et al., 1999). Conceivable are also seasonal influences or, as mentioned before, the longer storage period of the meat.
For liver tissue there was no obvious difference between total fat and phospholipid fraction, so that in general the results from the first trial were confirmed again.

BLUNK et al. (1992) had performed an experiment also with cattle of German Fleckvieh breed, as well as similar feeding and slaughter weight. The order of major fatty acids was the same, just the percentages were varying slightly with oleic and arachidonic acid being higher and linoleic acid being lower than in the present study. Nevertheless, the summarised values for classes of saturation were also all about 33 %. The results by BOYLSTON et al. (1995) and ZEMBAYASHI & NISHIMURA (1996) were less comparable, probably due to differences in breed, feeding and sex. The first found higher concentrations of MUFA in combination with low contents of SFA and PUFA and the latter, on the contrary, determined higher SFA and lower MUFA contents. In general, the amounts of single fatty acids were not in agreement with the results from this study.

Serum fractions also showed great differences in comparison to the previous study. The triglyceride fraction, in contrary to the first Young Bulls experiment, consisted mainly of SFA (60 %, including 30 % stearic and 23 % palmitic acid), whereas in the previous experiment the SFA content was only 38 % due to a stearic acid concentration of only 14 %. On the other hand, oleic acid was measured to be 15 % (instead of 20 % in the previous trial) and linoleic acid to be 5 % (instead of 13 %), so that also a significant lower content of PUFA was detected. This strong discrepancy is very difficult to explain, as the experiment was planned to be equal (concerning animal breed, sex and feeding) except for the amount of supplementation with α-tocopherol. It was not expected that the frozen storage could change the pattern to such an extent. Unfortunately, no studies have been performed on this subject.

The phospholipid fraction contained besides 30 % stearic acid also 25 % linoleic acid and about 12 % of each palmitic and oleic acid, which was in agreement with the values determined in the Young Bulls experiment I.
The cholesterol ester fraction was characterised by a high content of 73 % PUFA (caused by 65 % linoleic acid), which was higher than in the trial before (63 % and 56 %, respectively). Taking into consideration that the present samples were stored longer in frozen condition, this is an adverse result to decreases in PUFA content in other tissues and should turn down the above described theory of degradation of PUFA during storage. The contents of SFA were lower instead, due to palmitic acid (6 % instead of 9 %) and stearic acid (3 % instead of 5 %). The MUFA content of 12 % is mainly represented by oleic acid and similar to the one measured in the first trial.

In the case of serum fractions again no other results seem to be available from other authors. As HECKER et al. (1975) had not separated the different fractions of serum, a comparison was already not possible with the data from the first trial. The results of human plasma by FRITSCHE et al. (1998) were comparable to the previous study concerning phospholipids and cholesterol ester fractions, so that the values for phospholipids will also be concurrent to this trial. Consequently, the cholesterol ester fraction was not in agreement and the triglyceride fraction was also not comparable. But as this was human plasma, the comparison must not be taken seriously anyway.

As could already be seen in the first Young Bulls experiment, the fatty acid patterns did not differ significantly within tissue types in relation to α-tocopherol supplementation, so that it was concluded that α-tocopherol did not help PUFA to be incorporated. But when subjecting the data to Multivariate Data Analysis, a certain association between distinct fatty acids and the supplementation of α-tocopherol became visible in the loading plots. In MLD the influence was lowest. Only for long-chain PUFA in total fat a certain tendency of increase with supplementation was detected. More pronounced (but still very small when looking at the original data) were effects in MST, serum and liver. With increasing amount of α-tocopherol supplementation, the following concentrations increased: single PUFA as well as the total PUFA content in phospholipids of MST, single SFA and total SFA in the triglyceride fraction of serum, and short-chain fatty acids in kidney tissue. On the contrary, associated with the control group were several unsaturated fatty acids as well as total MUFA and total PUFA content in the serum triglyceride fraction, single SFA and total SFA content in the phospholipid fraction of MST, as well as total MUFA in liver tissue. In order to see the scores for the single animals refer to appendix 8.7.3.

4.3 Experiment III: Influence of Feeding Type for Steers

The previous experiments have demonstrated that dietary supplementation with high levels of the antioxidant α-tocopherol was effective in improving beef quality with respect to colour stability, water holding capacity and fat oxidation stability, but did not change fatty acid patterns to a significant extent. However, these animals did not have access to fresh forage which contains high levels of endogenous α-tocopherol and also higher concentrations of readily oxidisable ω-3 unsaturated fatty acids (VEGA et al., 1996). Cattle cannot synthesise α-tocopherol and normally obtain it by consuming pasture. α-Tocopherol is abundant in whole cereal grains, particularly in germ. Green forage and other leafy materials, including good-quality hay, are very good sources (MCDOWELL et al., 1996; SMITH et al., 1996). Additional advantages of pasture based feeding are the commercial use of the increasing numbers of permanent grassland, and grazing being a positive quality criterion to consumers.
Therefore this third trial was designed to quantify the effect of three feeding strategies (including pasture, silage and a mixture of both) towards α-tocopherol content and fatty acid composition of MLD, liver, kidney fat and serum.

4.3.1 Experimental Design

Thirty-eight male calves (German Fleckvieh = Simmental), castrated at 120 days of age, were divided into three groups, according to tab. 7: The first group received indoor feeding with maize silage *ad libitum* plus concentrate, comparable to the young bulls experiments (see 4.1.1). The other animals in the beginning received the same treatment. They were fed pasture (grazing grass and clover) for 169 days, received winter indoor feeding (restricted amounts of grass, maize silage and hay) for 162 days and then were split up again. A second group was left grazing for the last 165 days, whereas the third group was fed a combined ("mixed") diet: grass and clover over a period of 72 days, and for the last 66 days the animals were fed maize silage, comparable to the first group. None of the groups received additional α-tocopherol supplementation.

Table 7: Steers "Feeding Type", divided into feeding groups
(S = silage, M = mixed, P = pasture, n = number of animals)

	Group "silage"	group "mixed"	group "pasture"
feeding conditions	Maize silage (plus concentrate)	pasture 72 days / silage 66 days	pasture (grass and clover) 165 days
number of animals	n = 10	n = 11	n = 17

The final overall mean life-weight at slaughter was about 600 kg. The steers were kept in groups of five to six animals but feed intake was measured individually. The silage (plus concentrate) contained about 25 mg α–tocopherol/kg dry matter, the pasture diet about up to 60 mg per kg.
Animals were raised and kindly supplied by the Institute of Animal Nutrition Physiology of the Technical University of München (Freising-Weihenstephan, Germany).

4.3.2 Slaughter and Sampling Procedures

The procedures were done as described in 4.1.2. For measurements of α–tocopherol contents and determination of fatty acid patterns were taken blood samples as well as samples from the liver, kidney fat and the foreloin (*Musculus longissimus dorsi* - MLD).
α-Tocopherol contents were determined once for every single animal. For fatty acid pattern only six samples per group were taken randomly and their tissues were analysed once.

4.3.3 Results from Co-operating Institutes

The average daily life-weight gain was highest for the silage group (1020 g; 385 days to slaughter), followed by the mixed (820 g; 469 days) and the pasture group (740 g; 496 days). Animals from the silage group had a significant higher fat class, contained more tallow and nearly twice as much intra-muscular fat in MLD (4.3 %, compared to 1.6 % in the pasture and mixed group). However, subjective parameters like marbling and organoleptic qualities (juiciness, tenderness and aroma) were also markedly higher with the higher fat content and therefore positively correlated with the intensive silage feeding system (SCHWARZ et al., 1999). Unfortunately though, effects on colour stability, fat oxidation and water retention were not determined for this experiment.

4.3.4 Results and Discussion of α-Tocopherol Contents

The α-tocopherol contents were significantly higher for the pasture treatment than for the other treatments. The differences between the tissues were the same as already detected in the first Young Bulls experiment: α-tocopherol contents were decreasing in the order of liver, kidney fat, serum and MLD. Liver tissue showed also the highest deviation.

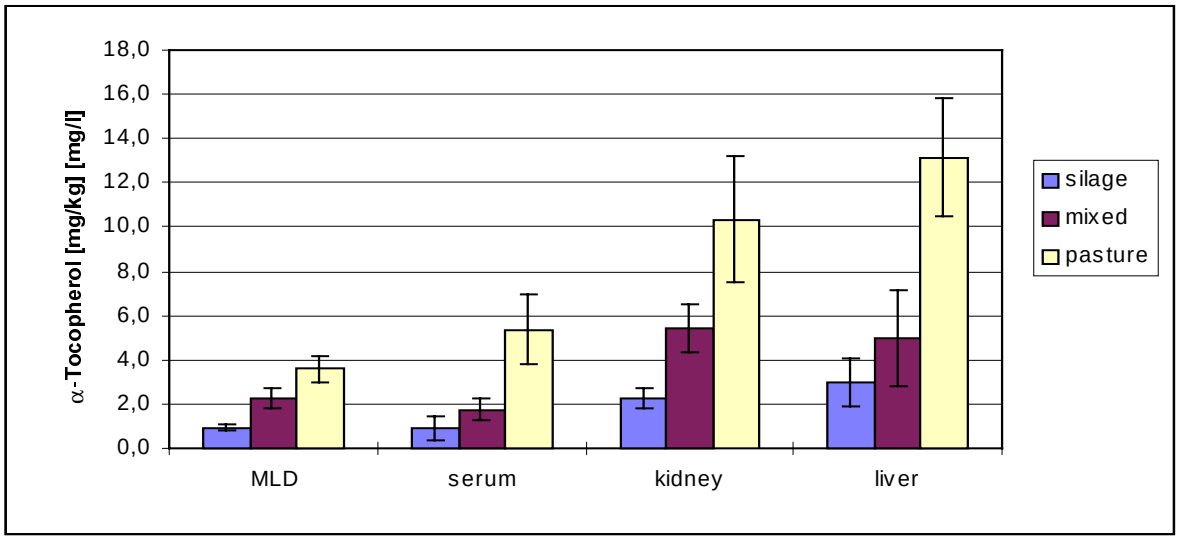

Figure 13: Steers "Feeding Type" - α-tocopherol contents in different tissues dependent on feeding (n = 10-17)

According to fig. 13 (also refer to appendix 8.7.1) α-tocopherol contents of ca. 0.9 mg/kg (MLD, serum), 2.3 mg/kg (kidney fat) and 3.0 mg/kg (liver), respectively, were determined in silage fed animals. Compared to the control groups of the two previous experiments these steers already showed higher values (with the slight exception of group C 120) than the bulls. As all animals were of the same breed, this difference could result from the different sex, maybe combined with the higher fat content that the steers had (4.3 % compared to ca. 1.6 % for the bulls). Other authors have published values for steers of about 1.6-3.0 mg/l for serum and 1.6-8.6 mg/kg for liver (ARNOLD et al., 1992, 1993a,b; GARBER et al., 1996). The high deviation for liver tissue is apparently confirmed by these data.

Feeding grass and clover resulted in α-tocopherol contents that were about four times higher: 3.6 mg/kg (MLD), 5.4 mg/l (serum), 10.3 mg/kg (kidney fat) and 13.2 mg/kg (liver), respectively. These values even exceeded those for group B 120 of the first Young Bulls Experiment, except for liver. VEGA et al. (1996, 1997) performed some comparable studies: different crossbred steers were fed either ryegrass or hay plus concentrate or grass silage. Those steers fed hay reached only a level of 1.9 mg/kg MLD, but for the others similar MLD contents than the one from the present trial were determined: 3.4 and 3.1 mg/kg, respectively. LYNCH et al. (1999) determined 2.3 mg/kg in MLD, when analysing Friesian cattle that were fed with barley and grass silage. Unfortunately in both publications no other tissues were analysed. Also LIU et al. (1995) described that cattle that grazed good-quality pasture had higher concentrations of α-tocopherol in skeletal muscle than cattle fed an unsupplemented high-concentrate diet or cattle not given access to fresh grass. HARTFIEL (1995) detected a seasonal influence, as young cattle on pasture had lower α-tocopherol contents in blood between autumn and spring. As well as MCDOWELL et al. (1996) found the α-tocopherol content of milk to be considerably influenced by α-tocopherol concentration in feedstuff. There can be a five-fold seasonal difference, due to pasture, which is rich in tocopherols and leads to an increase in milk's tocopherol content during summertime compared to those rather poor sources of winter feed such as silage.

The amounts for the mixed group (steers moving from pasture to silage) were, as expected, in-between the values for the other feeding groups: 2.3 mg/kg (MLD), 1.8 mg/l (serum), 5.4 mg/kg (kidney fat) and 5.0 mg/kg (liver) and comparable to the results from group A 120 or D 180 of the Young Bulls Experiments. Contents for serum and liver were in this case striking low, which confirmed the statement that those two are representing the immediate nutritional supply, as described for the previous trail. ARNOLD et al. (1993a) had performed a similar experiment with Holstein steers which were placed on grass-legume pasture for 86 days. Two days after they were removed from pasture, they were initiated on a maize treatment (comparable to all other ARNOLD experiments) without further α-tocopherol supplementation (196 days). The resulting values for α-tocopherol were 1.6 mg/kg in *lumborum* muscle, 2.1 mg/l in serum and 3.2 mg/kg in liver. Grazing elevated concentrations of α-tocopherol but to a lesser degree than in the steers fed supplemental α-tocopherol (2000 mg/day). Depletion of α-tocopherol was complete within six weeks for plasma and liver but required 12 to 18 weeks for muscle tissue.

Serum has mainly a transport function in the body, therefore the α-tocopherol content in the animals fed grass and later silage had dropped 100 days after change of feed down to almost the basic level. Also in the liver the incorporated α-tocopherol was reduced after 100 days because of metabolic processes. Kidney fat, on the other hand, has a storage function and therefore the α-tocopherol content was decreasing more slowly. PARKER (1989) and NOCKELS et al. (1996) confirmed this theory by describing that (in rats and guinea pig) rapid depletion rates after withdrawal of α-tocopherol from the diet were seen in serum, liver, lung, and brain, slower ones in skeletal and heart muscle and very slow rates in adipose tissue. Elevated plasma levels were not maintained, and dropped about 50 % four weeks after returning to a non-supplemented diet. Still the levels four weeks later were approximately 3-fold higher than the starting levels. In their case, the return of plasma α-tocopherol lagged behind those of the tissues (with exception of adipose tissue), possibly indicating mobilisation of tissue α-tocopherol into the plasma.

Conspicuous are the high α-tocopherol contents that were reached for the pasture group when considering the relative low α-tocopherol content determined in the feed (ca. 60 mg/kg). One possible reason is that the determination in the feed was not correct because of not quantitative liberation from plant tissue. The α-tocopherol content should have been much higher, as α-tocopherol content in forage is affected by stage of maturity at the time of forage cutting and the time from cutting to dehydration. But still with estimated double or triple content the effect would be higher compared to 2000 mg/kg that were fed in the Young Bulls Experiment I. A second analytical problem could be the poor stability of all naturally occurring tocopherols, so that substantial losses of α-tocopherol activity may have occurred in feedstuff when processed and stored (e.g. until analysis). Another possible reason is, as some studies have indicated, that the "natural" D-form results in higher serum and selected tissue α-tocopherol concentrations than the chemically synthesised form (DL) when administered on an equal IU basis (HIDIROGLOU et al., 1988; MCDOWELL et al., 1996).

α-Tocopherol supplementation of grazing steers improved meat quality despite high endogenous levels obtained from grass and the smaller incremental deposition compared with supplemented concentrate fed animals. There was evidence of differences in meat quality between cattle fed grass or concentrate. Grass-fed animals tended to have higher α-tocopherol levels but had lower colour stability than their concentrate-fed counterparts. Steaks of the α-tocopherol supplemented animals were redder and developed lower TBA values. Panellists detected higher abnormal flavour and scored flavour liking lower for meat from grazing animals. Beef flavour was not affected by dietary supplementation with α-tocopherol. Redness declined less rapidly in the supplemented groups and they had significantly lower TBARS levels (VEGA et al., 1996, 1997; WOOD & ENSER, 1997). Unfortunately there were no experiments concerning oxidative stability performed with meat from this trial.

Also a convincing argument for high α-tocopherol levels in pasture fed animals is the assumption that other components from grass and clover have a synergistic effect on α-tocopherol incorporation into tissues and its antioxidant activity, respectively. One possibility would be e.g. ascorbic acid (vitamin C), which has already been mentioned in literature.

Ascorbate is found in meat at maximal levels of 140 µM. The pre-slaughter injection of ascorbate delayed oxidation in beef, as well as exogenous addition of ascorbate. However, ascorbate may act either as a pro- or antioxidant in foods. It is possible that the antioxidant activity of some lipid-soluble chain-breaking antioxidants might appear to be greater in systems containing ascorbic acid. Vitamin C extends its antioxidant protection by reducing the semi-stable tocopheroxyl radical, resulting in the regeneration of the metabolically active form of α-tocopherol (KITTS, 1997). But vitamin C also contributes its electrons to other oxidised neighbouring species, such as e.g. metal ions, singlet oxygen, hydroxyl or hydroperoxyl radicals. A proposed model of the oxidation-reduction relationships in beef with emphasis on α-tocopherol, vitamin C, phospholipids and myoglobin is published by SCHAEFER et al. (1995). These interactions of vitamin C, α-tocopherol and other antioxidants present in (or added to) foods are very important, as they are providing a more effective means for inhibition of lipid oxidation than either one of the vitamins alone (YIN et al., 1993), not only in preventing rancidity but also in maintaining the nutritional quality of the food by keeping high levels of the antioxidant vitamins (HALLIWELL et al., 1995).

The importance of this interaction in meat systems has received limited attention. The existence of a synergistic relationship between these two vitamins has only been addressed in few meat systems. OKAYAMA et al. (1987) dipped fresh beef loin steaks into solutions of ethyl alcohol containing either DL-α-tocopherol, L-ascorbic acid or a combination of both. TBA numbers of samples dipped into the ascorbic acid/tocopherol mixture were lowest after 9-13 days of storage. These results suggest that synergism with ascorbic acid enhanced the antioxidant activity of α-tocopherol in retail cuts of beef loin steaks. SCHAEFER et al. (1995) stated that plant material consumed by cattle provides sufficient vitamin E to meet their growth requirement, and synthesis of vitamin C by ruminant liver apparently satisfies the metabolic requirements for ascorbic acid. Nevertheless, supranutritional supplementation of α-tocopherol and *ante mortem* infusion of vitamin C to finishing steers can improve colour and lipid stability of beef (ARNOLD et al., 1992, 1993). The *ante mortem* activity of α-tocopherol and vitamin C continues to function in *post mortem* muscle (meat), where they have a critical role in maintaining quality in the food product. Dietary supplementation of α-tocopherol, and intravenous infusion of vitamin C immediately before harvest, are efficacious techniques for increasing the concentration of these vitamins in beef skeletal muscle. Meat with elevated levels of either and probably both of the antioxidant vitamins possesses greater stability of oxymyoglobin and lipid, which results in less discolouration and rancidity, respectively. *Ante mortem* nutritional intervention appears to be a promising approach for improving the quality of fresh meat products subsequently obtained from livestock. *Post mortem* application of vitamin C has been evaluated as a means for improving lipid and colour stability of beef. Applications of ascorbic acid to surfaces of intact muscle cuts though may be less effective than *ante mortem* infusion because the reducing agent is not likely to be in intimate contact with metmyoglobin.

It would be worthwhile to further investigate the interaction of dietary α-tocopherol level and ascorbic acid administered to muscle by feeding. Therefore further experiments were conducted at the Technical University of München, in which by supplementation with α-tocopherol and ascorbic acid separately and in combination this influence should be studied.

4.3.5 Results and Discussion of Fatty Acid Patterns

In this experiment not only differences between tissues could be detected, but also fatty acid patterns differed within the type of tissue in dependence on feeding. Therefore mean values of the feeding groups were listed in the appendix (refer to 8.7.2), but a total mean was not calculated. For oleic acid and linolenic acid the effect could be seen throughout all tissues and fractions. Contents of oleic acid were "decreasing" significantly when comparing the silage-fed group with the group fed grass; in most cases a stepwise lower content could be observed with the stepwise increase of grass and clover (from the silage to the mixed and further on to the pasture group). On the other side, linolenic acid was "increasing" most significantly in the same order. Also eicosapentaenoic, docosapentaenoic and docosahexaenoic acid showed their highest contents mostly for the group which was fed only grass and clover, but occasionally the highest content was determined for the mixed feeding group. The sum of PUFA did not show this correlation in such a clear way, but the presence of higher amounts of ω-3 PUFA and especially eicosapentaenoic and docosahexaenoic acid in forage-fed cattle is not surprising because certain browse and pasture contain elevated contents of these fatty acids (VEGA et al., 1996; MANDELL et al. 1997). On the contrary, docosatetraenoic acid and partly also arachidonic acid showed the opposite response. These tendencies could be detected in the total fat as well as in phospholipid fractions and especially pronounced in the serum fractions.

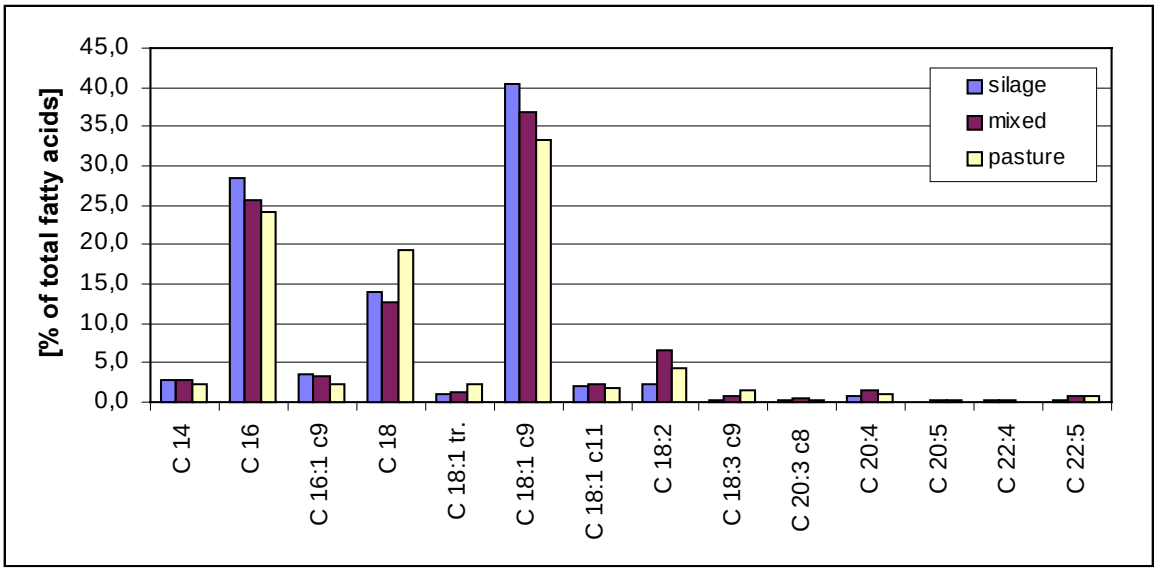

Figure 14: Steers "Feeding Type" – selected fatty acids of total fat in MLD depending on feeding strategy

In total fat of MLD (see fig. 14, also refer to appendix 8.7.2), oleic acid was the major fatty acid (33-40 %), followed by palmitic acid (24-28 %), stearic acid (13-19 %) and linoleic acid (2.3-6.6 %). Further myristic, palmitoleic, elaidic, vaccenic and arachidonic acid were determined in the 1-4 % range. Palmitic and oleic acid were significantly lower in forage-fed animals, while it was vice versa for stearic acid. Linoleic, linolenic acid and highly unsaturated fatty acids were mainly detected in those animals that had received pasture. Summarised, levels of 43-48 % SFA, 41-49 % MUFA and 4-11 % PUFA were calculated. For animals on the pasture

treatments was the proportion of PUFA more than twice compared to those animals with silage treatment, but even higher were values of the mixed group. Inversely, concentrations of MUFA became lower with feeding of pasture. The proportion of SFA did not differ extremely between treatments. A similar tendency (pasture vs. silage) was also found for fatty acids in all other tissues and fractions.

Compared to the other two experiments described before, the range for most fatty acids covered the values determined in the previous trials. Just values for linoleic and arachidonic acid were significantly lower, most obvious for the silage-fed group. As animal breed and feeding were designed to be comparable, only the difference in sex could be taken into account for this variation. In case of linoleic acid it could also be due to the higher intramuscular fat of the silage fed animals, because ITOH et al. (1999) found that levels of linoleic and linolenic acid decreased with increasing intramuscular fat content – they did not comment on arachidonic acid though. Concerning the favourable ω-6:ω-3 ratio in meat, grazing ruminants usually show the best values. The present pasture group actually has a value below 4.0, while the mixture group is slightly above the limit, but the silage-fed steers are far beyond it, similar to the Young bulls. A certain protection of PUFA against the saturating effect of the rumen occurs when grass-based or grain-based diets are fed, leading to relatively more ω-3 or ω-6 fatty acids, respectively (WOOD & ENSER, 1997).

There are few recent publications, in which the authors had performed feeding experiments with focus on comparing forage and grain feeding. ITOH et al. (1999) raised Simmental and Angus steers on annual or perennial pasture or on grain feeding. They could detect significant differences, which were greatest for comparisons between the grain and the two pasture groups, but there were also differences between the two pasture groups. Relative to intramuscular triglycerides of animals in the grain group, those of the two pasture finished groups contained more oleic (44-45 % compared to 41 %), linoleic (1.3-1.6 %/0.5 %) and linolenic acid (1.0 %/0.2 %), but less palmitic (26-27 %/30 %), palmitoleic (3.4 %/3.9 %) and myristic acid (2.6-2.9 %/3.4 %). Oleic acid was the major fatty acid, comparable to the present trial, but found in a much higher content for the pasture groups, which is not in agreement with the results from the present study. The other values and tendencies were comparable, except for stearic acid (13 %/14 %), which was not found to be significantly different by ITOH et al. (1999), but it was significantly higher for pasture in the present trial. The increase in linolenic acid with pasture can be explained with being the predominant fatty acid for leaf lipids, on the other hand linoleic acid should be major fatty acid in seeds such as maize grain and therefore be lower with forage-based feed. But also reports in the literature are inconsistent for same fatty acids, especially oleic acid and linoleic acid. Possible reason for the deviation in this case could be that carcass weights (500 kg) and intramuscular fat content (3-18 %) were very high. Further information on the fatty acid composition of the feed types were not collected / available, but such differences are the most likely explanation for the variations outlines above, as lipid in feed of cattle are not totally hydrogenated by bacterial action in the rumen (MANDELL et al., 1997).

This is also the main argument by WOOD & ENSER (1997), who propose that tissue fatty acid composition can be changed via the diet, which involves manipulation of rumen fermentation pattern. The fatty acid composition of concentrate (grain-based) and forage (grass-based) diets are quite different and therefore lead to different fatty acid compositions in tissues. In their study, steers finished on grass had higher concentrations of linolenic acid and all other ω-3 fatty acids than young bulls given a barley-soybean concentrate diet, which had higher concentrations of linoleic acid and all other ω-6 fatty acids. The concentrations of ω-3 fatty acids were doubled with pasture feeding, while linoleic acid and arachidonic acid increased ca. three-fold with concentrate. These results are explained by the fact that linolenic acid is the major fatty acid in grass lipids whereas cereals and the oil seeds used in concentrate diets are major sources of linoleic acid. A proportion of each of these has clearly avoided breakdown in the rumen. Of course, a certain effect of the different sex should also be considered.

CAMFIELD et al. (1997) studied crossbred steers, which were developed on forage (bermudagrass) and subsequently finished in a feedlot. In MLD they measured the following changes with duration (finishing): oleic (34-38 %) and palmitic acid (24-25 %) increased, when animals were taken from forage. These results, in terms of amount and development of fatty acid contents, were confirmed by the values measured for the experimental animals of the mixed group. On the other hand, stearic (18-13 %) and linoleic acid 1.4-0.5 % concentrations decreased significantly, as well as myristoleic, pentadecanoic, linolenic, eicosenoic, eicosatrienoic, eicosapentaenoic and docosapentaenoic acid dropped slightly about 0.1 % each, when the amount of concentrate in the diet was increased. The drop in most fatty acids could be confirmed with the present trial. Concerning linoleic and arachidonic acid it has to be mentioned though, that the silage-fed group actually had the lower results, whereas the mixed group contained the highest amounts. It was in agreement with the present trial, that SFA except linoleic acid increased in correlation with the duration that cattle were fed a high grain diet whereas most unsaturated fatty acids except oleic acid were higher in cattle that were not fed a high grain diet. Also VEGA et al. (1997) and MANDELL et al. (1997) proposed higher PUFA contents for animals fed pasture due to the elevated contents in the forage.

RULE et al. (1997) analysed MLD of crossbred steers fed with different feeding strategies for different lengths of time. The first group received maize and barley and were slaughtered very young. The second group was kept for half a year longer and received forage and later maize and barley, whereas the third group was kept another 6 months longer and fed maize and hay first, then forage and in the end maize and barley. The MLD SFA and MUFA changed little with duration. In the second group palmitic acid increased (26-29 %), while for the second and third group stearic acid decreased with duration (17-13 %), comparable to the mixed group of the present trial. Oleic acid increased for the first and third group (37-45 % and 40-47 %, respectively); the same tendency was found for the silage group in this study. In contrary to CAMFIELD et al. (1997), PUFA decreased and MUFA increased with time on feed, such as it was also determined in this present trial, just the values for MUFA were slightly lower. Concentrations for most other fatty acids were in the same range as the ones from the present trial, with only small differences in oleic, palmitoleic and arachidonic acid as well as SFA. MANDELL et al. (1997) concluded that nutritional factors also affected tissue fatty acids in cattle

(not only breed and sex), e.g. forage compared with grain feeding resulted in greater unsaturated fatty acids. And they stated that those steers backgrounded, followed by summer pasturing and then feedlot finishing, produced muscle with optimal ratios of hypocholesterolemic to hypercholesterolemic fatty acids.

Total fat of liver tissue contained only 10-14 % oleic acid and 11-14 % palmitic acid, but almost 30 % stearic acid and 7-11 % arachidonic acid. Except for linoleic acid (7 %), which showed the highest discrepancy to the previous trials, all other detectable fatty acids with a chain length up to C18 were again below 1 %. But some long-chain and PUFA were present to a higher extent: eicosatrienoic acid (4-7 %), docosatetraenoic acid (1-4 %) and docosahexaenoic acid (1-4 %). This led to 42 % SFA, only 14-18 % MUFA but 36-42 % PUFA, which was a result more similar to the second than to the first Young Bulls experiment.

Kidney fat on the other hand contained only 1-2 % PUFA in total fat, which was even less than in the second trial. Palmitic acid (24-28 %), stearic acid (26-33 %) and oleic acid (23-29 %) were the major fatty acids, their ranges covered again the values from the other studies. Myristic, palmitoleic, margaric, elaidic and vaccenic acid were again present with 1-4 %, and no long-chain fatty acids occurred. In this tissue the SFA content was determined to be 60-63 %, the remaining 31-37 % were MUFA.

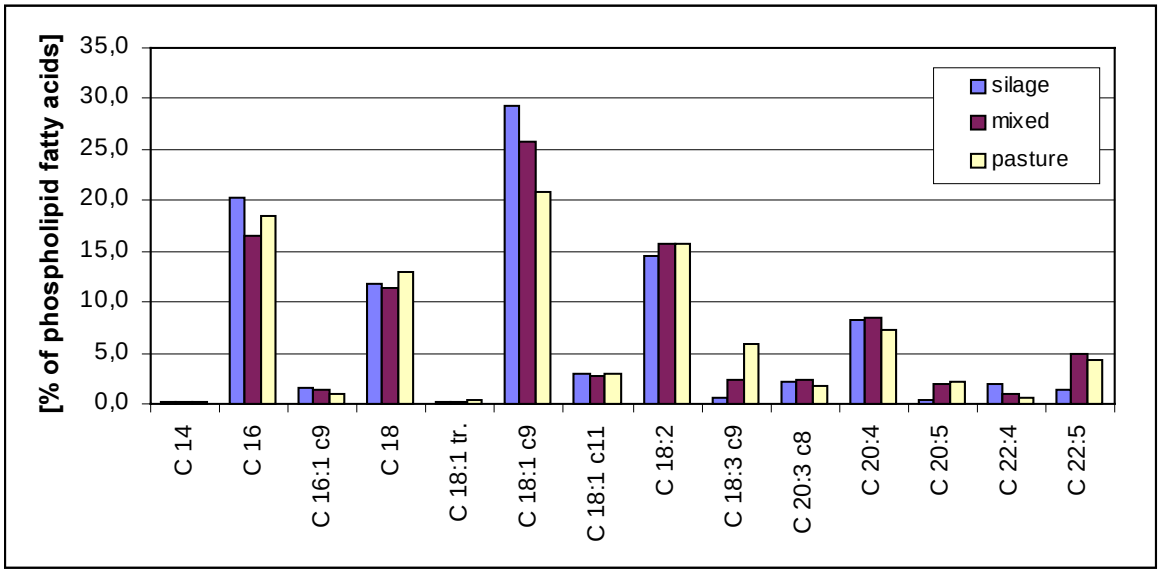

Figure 15: Steers "Feeding Type" – selected fatty acids of phospholipids in MLD depending on feeding strategy

The fatty acid distribution in the phospholipid fraction of MLD (see fig. 15, also refer to appendix 8.7.2) was also quite different from the results of the first study, but was closer to the ones from the second study. These tissues contained higher concentrations of oleic acid (21-29 % instead of 18 % in the first trial but 25 % in the second), but lower concentrations of palmitic acid (16-20 %, compared to 21 %/18 %), linoleic acid (15 % instead of 20 %/18 %), as well as stearic acid (11-13 % instead of 16 %/14 %). The content of ca. 7-8 % arachidonic acid was just slightly lower. Oleic acid was significantly higher in silage fed animals compared to those that had received pasture, while it was vice versa for linolenic, eicosapentaenoic and

docosapentaenoic acid. The other fatty acids were not significantly different. As a consequence, the MUFA content was between 27 % (pasture) and 35 % (silage; compared to 24 %/31 %), whereas the concentration of SFA dropped to 29 % in the mixed group (38 %/33 %). The PUFA content varied between 30 % (silage) and 38-39 % (pasture), compared to 33 %/34 % in the previous trials, mainly due to the higher contents of linolenic acid and the long-chain PUFA, except for docosatetraenoic acid. Surprisingly, it was not necessarily the values from the silage-fed group which matched the results from the second (or in some cases even from the first) trial, but very often the pasture-fed group or the mixed group came closer to the previous results. Therefore it was concluded, that not only the feeding but also especially the sex must have a strong influence on fatty acid composition and uptake/metabolism of fatty acids, respectively.

ITOH et al. (1999) also studied the influence of feeding towards the fatty acid pattern in the phospholipid fraction of *longissimus* muscle of Simmental and Angus steers. Again the effects were greatest between grain and pasture groups, but also significant differences were detected between annual and perennial pasture. The pasture group muscle contained less stearic (11 %/14 %), palmitoleic (2.5 %/4.3 %) and linoleic acid (11 %/12 %) and significantly more palmitic (18-19 %/14 %), linolenic (5.5 %/1.0 %), eicosapentaenoic (3.2-3.7 %/0.4 %) and docosapentaenoic acid (3.8-3.9 %/1.3 %), with the latter two being also significantly different between the two pasture groups. Most reports have indicated that levels of especially the last three fatty acids are lower for grain-fed groups, which was also in agreement with the present study. The other results are also comparable concerning the range, just that significance could not be confirmed for all the fatty acids named in the study by ITOH et al. (1999).

For liver tissue there were slight differences this time between total fat and phospholipid fraction, causing a higher PUFA content (38-43 %) together with a lower MUFA content (11-15 %) in the latter. The amount of SFA (41 %) remained the same, although palmitic acid decreased to 9 % and stearic acid increased to 31 %. The low content of 7 % linoleic acid was again the strongest difference to the previous trials, followed by rather low contents of oleic and arachidonic acid (8 %) in the pasture groups. From all tissues the liver phospholipid fraction was the one where feedings groups had the lowest influence on fatty acid composition. This result confirmed ALLEN & FOEGEDING (1981), who had explained that phospholipids contain a limited range of fatty acids, which is conservative. This range cannot be extensively influenced by diet or other environmental factors, because of the biological functions of phospholipids, which are in part influenced by fatty acid composition.

Serum fractions again showed great differences in comparison to the first study, but not so much compared to the second trial. The triglyceride fraction again contained mainly SFA (62 %, including 27 % palmitic acid – which was slightly more than in the second trial – and 26-29 % stearic acid – slightly less). Oleic acid was measured to be 15 % (the same as in the last trial) and linoleic acid to be 3-4 % (instead of 5 % in the last trial). The content of PUFA was about the same for the silage-fed group, but the pasture-fed groups reached higher levels compared to the last trial, as mentioned before.
The phospholipid fraction this time contained only 22-27 % stearic acid (30 % in the other trials), even less linoleic acid (14-16 %, compared to 25 %), the content of about 14 % palmitic

acid was instead slightly higher, but 11-15 % oleic acid were again in agreement with the values determined in the Young Bulls experiments. Obviously higher were again the results of long-chain PUFA, mainly in the pasture-fed groups, so that finally the sums of SFA (42-43 %), MUFA (13-18 %) and PUFA (34-40 %) were in the same range as in the other trials before, with the mixed group being closest to those results.
The cholesterol ester fraction was again characterised by a high content of 67 % (silage and mixed group) to 79 % (pasture group) PUFA (caused by 52 % and 44 % linoleic acid, respectively), which did not fit with any of the values from the trials before. Very remarkable in this case was the content of linolenic acid, which was extremely high (26 %) only in the pasture group, compared to all other values (ca. 3 %, also in the previous trials). From this result it seemed that the complete amount of this ω-3 fatty acid was collected in the cholesterol ester fraction. Compared to this, all other differences were just minor, but oleic acid (4-9 %) was the reason for some dissimilarity in MUFA content, whereas palmitic acid (6-8 %) and stearic acid (1-4 %) were covering the results from the previous experiments.

Unfortunately, for all other tissues and fractions except MLD no data seem to be published in order to compare the obtained results with those by other authors.

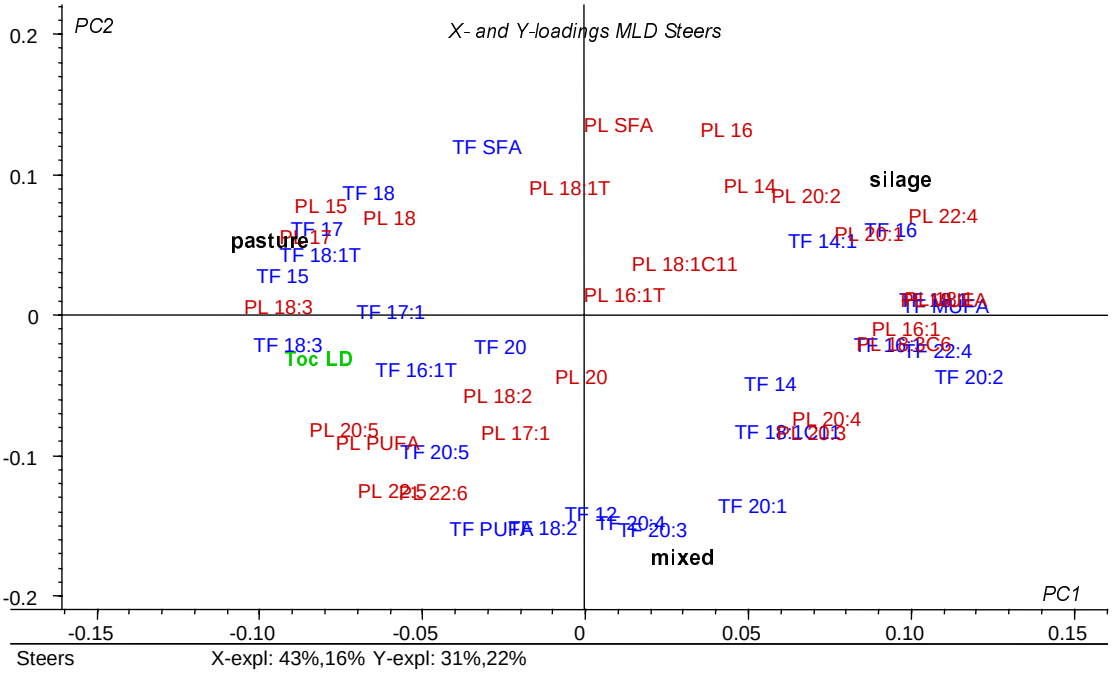

Figure 16: Steers "Feeding Type" – PLSR2-plot for MLD (TF = total fat, PL = phospholipid fraction)

The loadings plot for MLD of steers (see fig. 16, also refer to appendix 8.7.3 scores plot), as well as the corresponding ones for the other tissues, mainly confirmed the general conclusions made in the opening of this chapter. They showed the clearest pictures for this present trial compared to the two others. The probability of explanation was highest and in the scores plot three distinct feeding groups turned up as clusters. In MLD certain SFA of the total fat and the phospholipid fraction were most related to pasture feeding, although the content for SFA in both fractions was indifferent, located between pasture and silage, but not close to the mixed feeding. Also the PUFA contents were not very specific, but they were located between mixed and pasture

feeding, probably due to the contents being sometimes highest in the pasture and sometimes in the mixed group. Only docosatetraenoic acid was associated obviously more towards the silage group, as well as arachidonic acid in the phospholipid fraction tended to the same direction. Many MUFA in both fractions were orientated towards the silage group, except for C17 and trans-fatty acids, which were rather located toward the pasture feeding.
Liver values looked very much alike the pattern for MLD, while in serum the PUFA contents in all fractions were very closely associated with pasture feeding. A very distinct picture, though, was yielded for kidney tissue, in which not any fatty acids were associated to the mixed group, but most fatty acids split up either towards the silage or towards the pasture feeding. For example, all MUFA with even number of carbon atoms, except for trans-fatty acids and vaccenic acid, tended towards the silage feeding, whereas SFA, except for myristic and palmitic acid, were orientated towards pasture feeding.
In confirmation of the explanations made in the discussion of α-tocopherol content, it was shown by Multivariate Data Analysis that liver and serum α-tocopherol contents were actually located closer to pasture than levels in MLD or kidney fat, as both (liver and serum) represent the immediate nutritional status and were therefore relatively higher than the storage tissues, which need to accumulate such high amounts over a long period of time.

In comparison to the Young Bulls experiments for the first time elevated contents of (single) PUFA could be detected in association with raised α-tocopherol contents. This fact can not only be attributed to α-tocopherol itself, because in that case the other experiments should have shown an effect as well. The increased incorporation and improved protection of PUFA could then be due to the different fatty acid patterns in feed or to a synergism of α-tocopherol with other components of the feed as explained above.

5 QUALITY OF SALAMI

Previous experiments showed that nutrition in general and especially the supplementation with α-tocopherol had strong influence on α-tocopherol contents in pork and beef. Elevated levels of this antioxidant had positive effects towards meat in terms of retarded oxidation, resulting in a higher lipid and colour stability.
Concerning meat products, mechanical processes such as e.g. deboning, shredding and mixing disrupt the muscle structure and integrity of membranes and increase the surface exposed to oxygen and other oxidative catalysts (e.g. enzymes, haem pigments, metal ions; ZANARDI et al., 2000). To prevent from oxidation processes, some kind of preservation has to be applied.
Post mortem antioxidant addition for control of lipid oxidation may be scrutinised as concern for meat additives increases. Additionally, discrepancies between dietary supplementation and *post mortem* addition were detected, such as endogenous α-tocopherol improved pigment and lipid stability much better than exogenous α-tocopherol (MITSUMOTO et al., 1993). This can be attributed to the degree of subcellular incorporation of the tocopherols into phospholipid membranes of the tissues. Dietary supplementation allows uniform tocopherol incorporation into phospholipid membranes where it can effectively inhibit the oxidative reactions at their localised sites. In contrast, the distribution of the tocopherols during *post mortem* addition is not uniform, remaining outside of the protective cellular membranes. Evidence for antioxidant protection by exogenous α-tocopherol was even inconsistent: when added exogenously to meat products, α-tocopherol has demonstrated antioxidant activity, prooxidant activity and no effect. Therefore, dietary α-tocopherol supplementation in feed ensures that the nutrient is placed where its physiological action will be maximised and may produce meat products with less need for additives (ARNOLD et al., 1993b; LIU et al., 1994; FAUSTMAN, 1995).

The following trials were designed to study the effect of α-tocopherol supplementation in meat products, which were produced with meat and backfat from the experimental growing-finishing pigs. The main focus was set on stability against oxidation, so that besides α-tocopherol content and fatty acid composition different analytical parameters of lipid oxidation were measured (partly at the Fachhochschule Lippe, Lemgo, Germany). Salami was chosen as a meat product, which can be studied over a relative long storage period with only a small risk of microbiological spoilage. Oxidation processes are especially important for the quality attributes of this product both in a negative (rancidity, warmed-over-flavour) and in a positive (flavour) way. Therefore, fatty acid profiles of neutral lipid and phospholipid fractions are interesting as they are important precursors of beef flavour and off-flavour since they are the primary source of carbonyl compounds upon heating (IGENE & PEARSON, 1979; LARICK et al., 1989).

5.1 Experiment I: Influence of α-Tocopherol Content of Meat

Salamis were produced from meat and backfat of pigs, which had received three different levels of α-tocopherol supplementation. The production of Salami was performed corresponding to normal procedures as much as possible, but simplified e.g. in view of ingredients. The most important ingredients, however, such as curing salt premix, sugar, pepper, ascorbic acid, smoke and starter cultures, were added.
In order to force a certain oxidation process, meat was divided into two halves. The first half was used for Salami production according to the normal process, whereas the second half was prepared with a different choice of starter cultures. A rather common choice is the combination of *lactobacillus* and *staphylococcus* species. *Lactobacillus* bacteria cause an acidification, which is necessary together with other additives for ripening. Additionally the oxygen content is decreasing, so that oxidation processes will be retarded. On the other hand *lactobacillus* bacteria produce peroxides, which will be destroyed by *staphylococcus* bacteria. (LIEPE, 1971). Consequently, in the second batch only *lactobacillus* bacteria were added, so that the influence of α-tocopherol could be studied on Salami with elevated risk of oxidation processes.
Oxidative changes in muscle foods are generally quantified by the measurements of primary or secondary degradation products. TBARS are widely used as a measure of secondary oxidation products and it is accepted that they correlate well with sensory scores of oxidised and warmed-over-flavour in muscle foods. (BUCKLEY & MORRISSEY, 1982). On the other hand, the method provides only a sum of a variety of possible reaction products and additionally it is very susceptible to any kind of interferences or ongoing oxidation processes (FERNÁNDEZ et al., 1997). Therefore, a dynamic headspace method was developed and validated in order to identify and quantify single volatile compounds, e.g. hexanal as the main indicator for lipid oxidation.

5.1.1 Experimental Design

500 pigs (of the German Hybrid breeding programme) were selected at 30 kg of life-weight and were fed an extensive diet on the basis of grains and soya coarse meal up to live weights of 110 kg. The feed covered the daily requirements of trace elements, amino acids and vitamins.
Half of the animals (see tab. 8) received no additional α-tocopherol (the control ration in itself contained about 40 mg α-tocopherol per kg dry matter) for the first period of feeding. The feed for the other half of the animals contained 100 mg α-tocopherol (supplemented as α-tocopheryl acetate) per kg feed and pigs were continuously fed with this amount until slaughter. But the control group was split again after 50 days, one half continuing with no α-tocopherol being supplemented and the third group receiving 200 mg α-tocopherol per kg feed for the last 96 days prior to slaughter.

Table 8: Pigs, divided into feeding groups (n = number of animals)

	control group (n = 125)	Group 100 (n = 250)	group 200 (n = 125)
50 days	ca. 40 mg/kg feed	ca. 100 mg/kg feed	ca. 40 mg/kg feed
96 days	ca. 40 mg/kg feed	ca. 100 mg/kg feed	ca. 200 mg/kg feed

The animals were raised and kindly supplied by the "Erzeugergemeinschaft Osnabrück" (Georgsmarienhütte, Germany).
The pigs were slaughtered under normal conditions at the slaughterhouse of the "Erzeugergemeinschaft Osnabrück". Those parts needed for the production of Salami were sent to the Department of Meat Technology of the Fachhochschule Lippe (Lemgo, Germany).

5.1.2 Production of Salami

For each Salami production a pool of 10 animals was used. After frozen storage for about one week, 25-kg-batches were produced with a basic composition of 50 % pork shoulder and 30 % backfat, both derived from the animals of the trial, as well as 20 % beef (supplied as well from the "Erzeugergemeinschaft Osnabrück"). An addition of 2.8 % curing salt premix, 0.6 % glucose, 0.5 % sodium ascorbate, 0.25 % ground white pepper and 0.10 % starter cultures completed the recipe.
Sausages were partly ripened under "normal" conditions (starter culture Duploferment 66®, containing 50 % *lactobacillus plantarum* and 50 % *staphylococcus carnosus*; purchased from Rudolf Müller & Co, Gießen, Germany) or under "special" conditions (containing only *lactobacillus plantarum*); the second type was supposed to yield a type of Salami with higher susceptibility against oxidation.
The meat was closely trimmed, coarsely ground, mixed and reground. The ground pork was then mixed and weighed into 25 kg portions and the listed ingredients were added.
After smoking, the Salamis were left for ripening (20 °C) for one month; thereafter they were vacuum-packed and stored for up to 5 months in total.
The production of Salami took place in the Faculty of Food Technology, Department of Meat Technology at the Fachhochschule Lippe (Lemgo, Germany).

As samples for α-tocopherol and fatty acids analysis were taken the raw mixture of Salami ingredients (week 0) and Salami sausages periodically after 2, 4, 11, 16 and 21 weeks of storage. The samples were stored in the freezer until analysis. α-Tocopherol determinations were made on duplicate 2 g samples (according to the method for meat, refer to appendix 8.1.1), whereas fatty acid compositions (refer to appendix 8.2.1) were calculated from a single 5 g replicate which was injected twice.

5.1.3 Results from the Co-operating Institute

During storage of Salami peroxide value, thiobarbituric acid value and acid value did not differ significantly between groups in correlation to supplementation of α-tocopherol. Changes in acid number as well as physical parameters such as water content, weight loss, pH value, water activity, firmness and colour were comparable to Salamis processed under normal conditions. The peroxide value in the control group reached a maximum of 0.7 in the fourth week and dropped thereafter again. The tendency was the same for the other feeding groups, just with a maximum of 0.6 and 0.4 in the 100- and 200-mg feeding groups, respectively. Although the corresponding results for the Salami ripened under "special" conditions were higher (2.8-2.3), the rather low values indicate only minor changes in the fat fraction. Acid values were increasing gradually throughout the storage period (0.6-12), with the highest increase at the beginning. Differences, though, were not detected between feeding and ripening groups. This was expected, as the acid value is due to hydrolytic cleavage of fat rather than being a consequence of oxidation processes. Thiobarbituric acid values were highest in the first two weeks, which could be caused by either the analysis being rather unspecific or by a detection of components from pepper or smoke, which declined later due to their volatility. Weight loss, water content and water activity developed as it is known for normal ripening processes and showed no differences between treatments. There were also no significant differences concerning pH value, colour and firmness. In sensory analysis no development was detected between feeding groups or during storage. The results for the "special" ripening were lower, though, which might be due to the difference in starter cultures.

5.1.4 Results and Discussion of α-Tocopherol Contents

Salamis were highly significant ($p < 0.01$) different in α-tocopherol content (refer to appendix 8.8.1): with meat from the control group (no additional α-tocopherol; ca. 40 mg/kg feed) the Salami contained 3.0 mg α-tocopherol/kg. Meat of the second group (100 mg α-tocopherol/kg feed) led to Salami containing 4.4 mg α-tocopherol/kg whereas the meat of the third group (200 mg α-tocopherol/kg feed) resulted in Salami with an α-tocopherol content of 5.1 mg/kg. A five-fold amount of α-tocopherol in feed obviously caused the content in Salami to increase 1.7-fold, but already the group that received 100 mg showed a 1.5-fold higher value compared to the control group.

Many studies have investigated the effect of a α-tocopherol supplementation to animal diets on the meat α-tocopherol content, but there is not that much information available about meat products comparable to this present experiment.
Those values published on pork, varied quite substantially in the content of α-tocopherol in MLD and adipose tissue as a consequence of feeding (as well as breed, age, etc.). The highest value for MLD of a control group (3.2 mg/kg tissue) was reported by MONAHAN et al. (1990a), whereas in another experiment by MONAHAN et al. (1990b) only 1.2-1.5 mg/kg were determined. This range (1.0-1.7 mg/kg) was also confirmed by LANARI et al. (1995). But on the other hand

ASGHAR et al. (1991) and O´SULLIVAN et al. (1997) found only 0.5-0.8 mg/kg while LEONHARDT et al. (1997) as well as PFALZGRAF et al. (1995a) measured 2.5 mg α-tocopherol/kg. In the first Pork experiment (see 3.1.4) also rather high results of 2.1-3.2 mg/kg were determined.
For the corresponding adipose tissues the values differed even more. ASGHAR et al. (1991) determined only 1.2 mg/kg, MORRISSEY et al. (1996) already 5 mg/kg, LEONHARDT et al. (1997) 9.9 mg/kg and PFALZGRAF et al. (1995a) even 12 mg/kg.
For MLD of groups supplemented with 200 mg α-tocopherol the α-tocopherol concentrations were within a range of 4.1 mg/kg (PFALZGRAF et al., 1995a) and 7.9 mg/kg (LANARI et al., 1995). The analogous values for adipose tissue varied from 9.7 mg/kg (ASGHAR et al., 1991) up to 29.2 mg/kg (LEONHARDT et al., 1997). For a supplementation with 100 mg only a single value was available for MLD (2.6 mg/kg) and adipose tissue (3.2 mg/kg) (ASGHAR et al., 1991).
With a relation of 50 % meat to 30 % backfat the contents of 3.0, 4.4 and 5.1 mg/kg in the Salami of the control group and supplemented groups, respectively, were definitely within the range of these results. The fact that the increase of α-tocopherol was 1.5-fold for the Group 100, but only 1.7-fold for Group 200 can be seen as in agreement with ROSENBAUER et al. (1996), who stated that the concentration will only increase (asymptotically) up to a certain plateau.

The only values available for Salami were published by ZANARDI et al. (2000). They studied the effect of higher-than-usual α-tocopherol contents on colour features of processed pork. Heavy pigs are raised in Italy for traditional products such as fermented dry sausage (Salami). Salami α-Tocopherol contents increased significantly from the control group (3.9 mg/kg) to the groups supplemented with 6 % sunflower oil (7.8 mg/kg), 6 % sunflower oil plus 100 mg α-tocopheryl acetate/kg (9.7 mg/kg) and 6 % sunflower oil plus 200 mg α-tocopheryl acetate/kg (11.5 mg/kg). Already the control group had a higher α-tocopherol content, but that can be due to the longer feeding period as the Italian pigs were raised up to 160 kg while the experimental animals only reached 110 kg. But the values for the other groups were incomparable with the present trial, as the oil supplementation has probably had profound influence. The increase though from the group that received sunflower oil to the two supplemented groups was 1.2- and 1.5-fold, respectively, so it was also observed that the values approached a certain plateau. Unfortunately, no information was available when the Salami samples were taken and how α-tocopherol contents developed during storage.

A decrease during storage could not be observed in the present trial, when data were corrected for the loss of weight by drying. No substantial difference could be detected between the different ripening types; contents were only slightly higher in the group of “special” ripening.
As α-tocopherol was added to be present in the meat as an antioxidant, it was expected that contents would decrease during storage when it is reacting with radicals. But values remained almost constant and therefore either no oxidation processes were going on in the Salami or oxidation products (radicals) were scavenged by other antioxidants or α-tocopherol was regenerated by some other ingredients of the Salami. The first explanation seemed rather improbable, as there should be some oxidation processes within 5 months of storage, at least in the group with “special” ripening. But exactly the fact that this specially treated group also did not show any changes throughout the storage period indicated the presence of other antioxidants that either minimise oxidation reactions or regenerate α-tocopherol.

It was already described in chapter 4 (Beef Quality) that a synergistic effect of ascorbic acid (NJUS & KELLEY, 1991) might be considered as an explanation for the stable fat fraction. OKAYAMA (1987) e.g. demonstrated delay of lipid oxidation by dipping beef loin into a solution of α-tocopherol and ascorbic acid. But lipid oxidation is also delayed by already 20 mg nitrite/kg, which was added as curing salt premix. Nitrite plays an important role both in colour and flavour development and as a preservative. It has potent antioxidant properties: nitric oxide could inhibit the generation of ferryl ions and lipoxygenase and cyclooxygenase activities, as it can be liganded to ferrous ions and works as an electron donor and a scavenger of free radicals. In addition nitrite and by-products seem to interact with lipids, changing their susceptibility to oxidise (MORRISSEY & TICHIVANGANA, 1985; KANNER, 1994). Additionally, the volatile phenolic compounds in smoke (produced by cleavage of polyphenols such as lignin) might as well act as very good antioxidants, due to their ability to act as electron donors and stabilise the resulting free antioxidant radical by resonance (AHMAD, 1996). Finally, the ethereal oil of pepper shows antioxidant properties (MILBOURNE, 1987; PALIC et al., 1993).

LAUTENSCHLÄGER et al. (1996) produced raw fermented sausages and ham from pigs, which had received different amounts of α-tocopherol with the feed (2-45 g during the growing-finishing period). Even after 30 weeks of storage, the contents of α-tocopherol were still highly significant. Those products with higher concentrations should have an improved sensorial quality (FLACHOWSKY et al., 1997), which could not be confirmed by the present trial.

5.1.5 Results and Discussion of Fatty Acid Patterns

Although the determination of fatty acids composition in total fat is suggested as an indicator for declining quality of meat products (CAMERON & ENSER, 1991), changes could not be detected. Fatty acid patterns in total fat were comparable throughout all feeding and ripening groups and remained constant during storage.
Main fatty acids were oleic acid 40% and palmitic acid 23%, followed by stearic acid 12% and linoleic acid 10%. Palmitoleic acid was contained to about 3%, myristic acid to about 1.5% and linolenic acid to about 1%. All other fatty acids that had been detected were below 1%. The PUFA content of the Salamis was about 13 %, which was still acceptable concerning the risk of oxidation.

ZANARDI et al. (2000) had also determined the fatty acid composition of Salami batches, which were produced from heavy pigs in Italy with dietary supplementation of 6 % sunflower oil and 100 or 200 mg α-tocopherol/kg. Salamis were matured up to four months, but no information is given when the samples for analysis were taken. Concerning the result from this study, there should be no change during storage anyway. The value for oleic acid was higher with 45.6 %, but palmitic (22.6 %), stearic and linoleic acid (12 %) were very similar to the present trial. The other fatty acids palmitoleic, myristic, eicosanoic, linolenic, eicosadienoic, arachidonic, margaric and eicosatrienoic acid were also in agreement with the present results, as they were between 2.2 % and 0.1 %. No difference between the different α-tocopherol supplementation groups was

named. The resulting SFA (37 %) and PUFA (14 %) contents were comparable, just MUFA (49 %) were higher due to the elevated content of oleic acid.
CHASCO et al. (1993) investigated the influence of the curing of dry sausage (Salami) on chemical composition of lipids. They found, that initial fermentation is responsible for an increase in unsaturation, this being reversed later in the drying-curing process. The saturated/ unsaturated ratio raised in C16 and C18 fatty acids, which they attributed to lipolytic phenomena that produce fatty acids with a greater amount of linoleic acid than oleic acid. Oxidation processes particularly affected the unsaturated linoleic acid and oleic acid. In contrary to the present study, they found that free oleic acid was increasing from 0.45-4.05 % while total oleic acid was decreasing during the curing process.

KALLIO et al. (1998) examined changes in the proportions of the most abundant fatty acids during ripening and storage of a Salami-type sausage made of herring filets, pork and lard. They could detect a statistically significant increase in the proportions of minor PUFA (eicosadienoic, docosadienoic and docosatrienoic acid.) during the 4-week ripening period. During the 4-month storage period though the fatty acid composition of total fats stabilised, indicating a reduction in the activity of micro-organisms. Only the proportion of stearic acid increased significantly, which could also be seen in the present study. Fatty acid pattern of course were not comparable, as the oil from herring fillets contributes to it.
BOSI et al. (2000) studied Parma ham. They investigated that the dietary inclusion of α-tocopherol (0, 100 or 200 mg/kg acetate) did not (significantly) affect the neutral and polar fatty acid profiles, nor the one for subcutaneous fat. The pattern of the intramuscular neutral lipids was also dominated by oleic acid (47 %); a value similar to ZANARDI et al. (2000) but higher than the one from the present trial. The order and values of palmitic (20.3 %), linoleic (11.3 mg/kg) and stearic acid (10 %) were than comparable again, but levels for SFA (33.3 %) were slightly lower and values for MUFA (51 %) and PUFA (15 %) slightly higher.

Backfat containing PUFA contents of more than 15 % (or even 25 %) should not be used for the production of raw fermented sausages, as firmness and stability against oxidation are decreasing significantly above this level (FISCHER et al., 1990; STIEBING et al., 1993). Considering these limits, meat and backfat for the experimental Salami must have had PUFA concentrations that were adequate for this purpose, and therefore a basis for a high-quality product was laid.
Additionally, ingredients such as curing salt, ascorbate, pepper and smoke improved the quality (concerning stability against oxidation).

The fatty acid composition of phospholipids differed from the one in total fat, as it was also shown in the beef experiments (chapter 4), but differences in dependence on α-tocopherol supplementation could not be detected in week 0. The major fatty acid at the beginning was linoleic acid (25 %), followed by oleic acid (18 %), palmitic acid and stearic acid with each ca. 16 % and arachidonic acid (8 %). Further palmitoleic, eicosatrienoic, docosatetraenoic and docosapentaenoic acid were present in a range of 1.0-1.5 %, whereas the other fatty acids followed with less than 1.0 %. The resulting values for SFA, MUFA and PUFA were 33 %, 19-21 %, and 37-40 %, respectively.

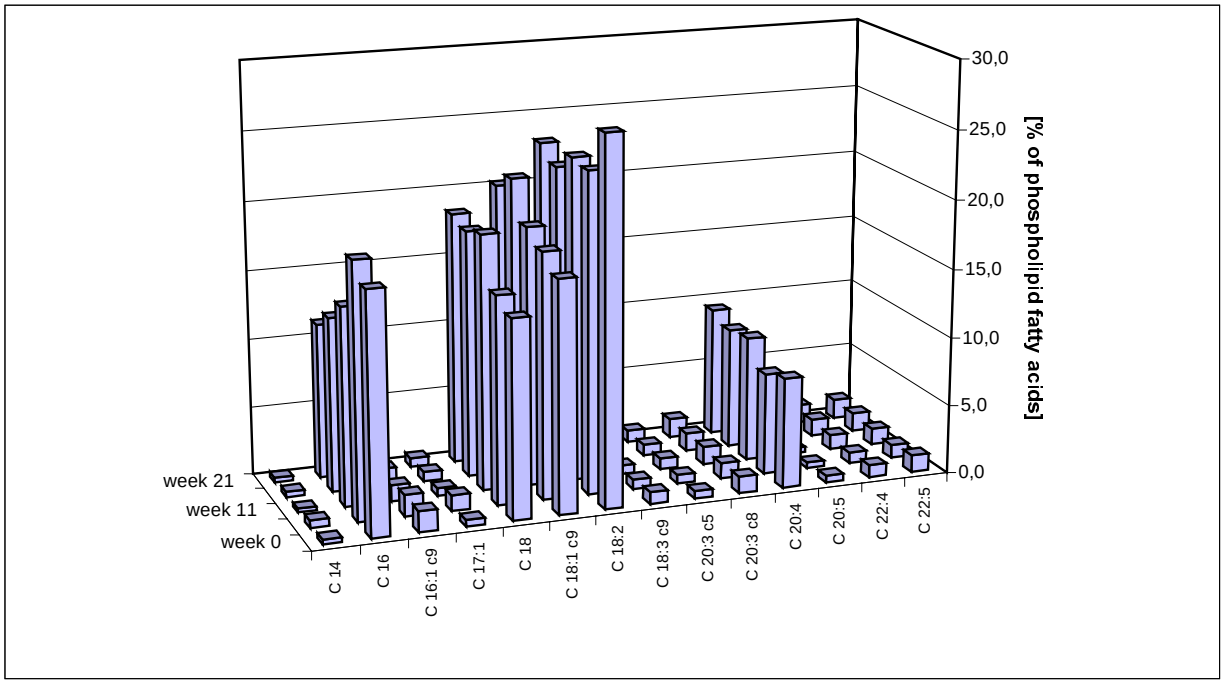

Figure 17: Salami I – alterations of selected fatty acids in phospholipids during storage (200 mg feeding group, "normal" ripening)

Also BOSI et al. (2000) could find no statistically significant effect ($p > 0.05$) of the treatments with α-tocopherol in the intramuscular polar lipids, but a tendency to higher MUFA and lower PUFA with increasing α-tocopherol supplementation. This cannot be confirmed with the data from this present study. Neither were the values for the single fatty acids in complete agreement: Linoleic acid was the main fatty acid with 26 %, followed by palmitic (18.8 %), arachidonic (18 %), oleic (16 %) and stearic acid (14.2 %). Very high was the PUFA content (47 %), while SFA (35.3 %) and MUFA (18 %) were closer to the levels in the present trial. Unfortunately, they did not study the changes of the fatty acid patterns during storage.

In contrary to total fat, alterations were detected in fatty acid patterns of phospholipids during the period of storage (see fig. 17, also refer to appendix 8.8.2). Especially for the "normal" ripening an inverse correlation was found between the decrease of linoleic acid content and the contents of α-tocopherol present in the Salami. Values decreased from 25.5 % to 20.5 % for the control group, but only from 24.8 % to 21.9 % for the second group (100 mg α-tocopherol/kg feed) and from 26.5 % to 23.4 % for the third group (200 mg α-tocopherol/kg feed). On the contrary, stearic acid and even arachidonic acid raised with about 4 % and 2 %, respectively. At the same time oleic acid increased, so that after two and a half months the contents of oleic and linoleic acid had approached each other and were about the same in the control group, while for the 200-mg-group the difference was still almost 5 % and the 100-mg-group was in-between. After this time also palmitic acid started decreasing, so that linoleic acid contents increased again.

The microbial conversion of fatty acids should be considered as one possible hypothesis for changes in the fatty acid pattern besides other enzymatic and non-enzymatic mechanisms. The fact that there was no overall trend with respect to changes in the fatty acid composition on a week by week basis, may have been due to both the sampling procedure and the normal variation

of the coarsely ground raw material mix, as it was also observed by KALLIO et al. (1998). In order to avoid such turns, it might have been an advantage to determine absolute fatty acids concentration instead of percentages of total fat content. In this case the results remained rather questionable, as it is surprising that PUFA, such as arachidonic acid, should increase with time of storage when oxidising substances were present. Nevertheless, the determined changes in fatty acid patterns of phospholipids did not influence fatty acid composition in total fat, as they are only present to about 0.5 % (CAMERON & ENSER, 1991).

The "special" ripening showed the same tendencies. Contents of linoleic acid were lower than in the other batches, as probably lipid oxidation processes were present to a higher extent due to the missing *staphylococcus* bacteria. The decrease of linoleic acid during storage was stronger the lower the α-tocopherol content was. This was in agreement with the "normally" ripened batches and was explained by a diminished prevention of the unsaturated fatty acid with less α-tocopherol being present.

5.1.6 Results and Discussion of Volatile Aldehydes (Hexanal)

Volatile aldehydes were isolated from raw Salami mixtures of the "special" ripening type (stored in the freezer for up to 6 months). Analysis was performed with the especially developed and constructed apparatus (refer to 2.3) for dynamic headspace sampling and thereafter identified and determined by headspace gaschromatography.

In the samples which were frozen immediately after mixing the ingredients only hexanal could be determined. In those samples that were analysed after 6 months of storage hexanal, heptanal, octanal and nonanal were identified. For hexanal an inverse correlation to α-tocopherol contents was found. In the control group the highest amount of 11.3 mg/kg was determined at the end of the storage period, for Group 100 still 3.5 mg/kg were analysed but Group 200 contained only 1.1 mg/kg. The degradation of PUFA, measured as content of hexanal as the most commonly used indicator for lipid peroxidation, was significantly retarded by supplementation of the feed with α-tocopherol.

It was very difficult to find publications that can be brought in for discussion of the results. There are authors that have analysed raw meat, cooked meat or stored meat, but not a fermented sausage, and especially not in the context of α-tocopherol supplementation. They might have either set their focus on identifying meat aroma rather than following the accumulation of oxidation products, or they have used other (old) methods, but did not determine hexanal and if they did, they did not calculate the absolute concentrations but gave only peak area results or relative amounts.
ZANARDI et al. (2000) analysed aldehyde contents of Salami and found that the long fermentation period had given the opportunity to lipolytic enzymes to break down triglycerides and fatty acids to a significant extent. They found hexanal to be the most abundant molecule, but the values determined were very low (0.3-0.4 mg/kg) and they were higher for the Salami which was prepared from meat of supplemented animals. But the variation in their analyses was very high

because of analytical difficulties. CHASCO et al. (1993) found 12-19 mg total carbonyls/kg in commercial products of Salami, with hexanal formation being predominant. They proposed that the carbonyl compounds increase in number throughout the curing phase, more than in the drying phase. PROCIDA et al. (1999) performed chemical analyses of aroma by the use of a static headspace method and they could identify 50 compound in Salami. Of oxidative origin, they detected hexanal, 2-hexenal, heptanal, octanal and nonanal in seven commercial Italian Salamis in very high concentrations (70-250 mg/10 g). LAI et al. (1995) evaluated hexanal contents of chicken nuggets treated with various antioxidants, e.g. oleoresin rosemary, after 6 months frozen storage with a similar technology to the present study. Hexanal levels ranged from 0.8 to 18.6 mg/kg, where the greatest hexanal concentrations were found in the control samples. Hexanal production could be significantly ($p < 0.05$) reduced 13 and 47 % by the addition of oleoresin rosemary (0.5 and 1.0 g/kg lipid, respectively). RAMARATHNAM et al. (1991) determined 12.7 mg hexanal/kg of fresh pork, but only 0.3 mg/kg in cured meat. SHAHIDI et al. (1987) measured 100 mg/kg cooked pork, but boiling cured pork (30 mg sodium nitrite/kg) led only to an amount of 3.0 mg/kg. FREEMAN & HEARNSBERGER (1993) detected pentanal, hexanal and nonanal in fish samples, which were stored at –20 °C for also about 6 months. After 4 weeks, though, already 80 µg hexanal/kg and 19 µg nonanal/kg were analysed. It was confirmed that cured meat products show lower hexanal contents, but unfortunately a direct comparison of the values was not possible, as data were obtained by different methods.

A reliable determination of the other aldehydes except hexanal was difficult, because the amounts were lower. Additionally, problems occurred for determination of aldehydes from Salami samples, because co-eluting peaks from supposedly pepper and smoking aroma were causing interferences. In order to solve this problem, a mass-spectrophotometer should be coupled to the GC for more specific detection. JOHANSSON et al (1994) studied fermented sausage after ripening and found a complex pattern of 100 substances, of which they could identify 88. The majority (in number and in concentration) belonged to the group of terpenes, followed by sulphur-compounds. Six aldehydes were found. They confirmed that many of the volatile compounds probably originated from smoke and seasoning (e.g. pepper), while others were a result of the activities of muscle enzymes and bacteria. SABIO et al. (1995) extracted volatile compounds from dry-cured Iberian ham (2 years of maturation), using dynamic headspace coupled to GC-MS, which enabled the identification of 40 compounds. These included 11 aldehydes, ketones, alcohols, n-alcanes, aromatic hydrocarbons, sulphur compounds, esters and terpenes, being the most abundant, in number and concentration, aldehydes and ketones.

It would also have been desirable to have measured aldehydes throughout the whole period of storage, as SHAHIDI & PEGG (1993) stated that hexanal content could decrease with longer storage time due to reactions with components of the meat or ongoing oxidation processes.

Nevertheless, from these results could be seen that in spite of curing, smoking and addition of ascorbic acid and pepper, which all have also antioxidant properties, an influence of α-tocopherol content towards fat stability of Salami is detectable.

Concerning the methods used, problems of static headspace GC with sensitivity and reproducibility are discussed widely in literature. These may be affected by sample equilibration time and temperature, and the potential for oxidation to occur during sample preparation and analysis. Difficulty of detecting low molecular weight molecules, sample particle size as well as the volume of sample may also have an effect (FREEMAN & HEARNSBERGER, 1993; SPANIER & BOYLSTON, 1994; WU et al., 1998). DALLA ROSA et al. (1994) measured for solid foods (e.g. raw ham) that the highest volatile concentration in the headspace also strongly depended on the availability of water. Techniques such as solvent extraction, vacuum distillation and high temperature steam distillation can be considered less adequate. These techniques yield good concentration of volatile compounds for GC analysis, but opportunities for heat and water-induced artefacts are great. The direct GC method with purge and trap thermal desorption technique has demonstrated a close correlation of taste panel flavour scores with individual volatile compounds observed. For efficient purging of tissue volatiles, provisions were made to heat the tissue to 50 °C while applying a vacuum to the downstream end of the glass liner (FREEMAN & HEARNSBERGER, 1993). A method that remains popular and extensively used despite its inadequacy is reviewed by FERNÁNDEZ et al. (1997): the TBARS test for lipid oxidation measurements. A detailed description of the different TBARS procedures, possible interferences and limitations of this technique are also given.

5.2 Experiment II: α-Tocopherol Supplementation and Rosemary Extract

An addition of plant oils to the feedstuff for pigs was consumer demand for a long time, as they associated health benefits with the resulting elevated contents of PUFA. Such an addition influences carcass quality and meat quality and composition only to a limited extent. But according to STIEBING (1993), backfat with high contents of PUFA (maximum 12-15%) is not appropriate for production of raw fermented sausages. As mentioned before, consistency of fat as well as oxidative stability of fatty tissues and lean meat is considerably decreasing. When they are supposed to be consumed fresh, the problem is not so serious, but these carcasses are hardly suitable for the production of raw fermented, dried, salted, and frozen meat and sausage products. Additionally, some unsaturated fats result in aroma deterioration. Preventively effective are some fats rich in middle-chain fatty acids and antioxidants (KREUZER, 1993).

Post mortem addition of antioxidants has proven less successful in limiting oxidative reactions in both beef and pork products (MITSUMOTO et al., 1993). The benefits of exogenous α-tocopherol addition at high concentration (100 and 1000 mg/kg) to frozen ground pork e.g. seemed limited to the first day of storage, beyond that there were no appreciable differences during the first 6 months. These conclusions, though, differed from those in studies involving dietary supplementation of livestock prior to slaughter which have found that α-tocopherol can significantly extend product quality (BUCKLEY & CONNOLLY, 1980; BUCKLEY et al., 1989).

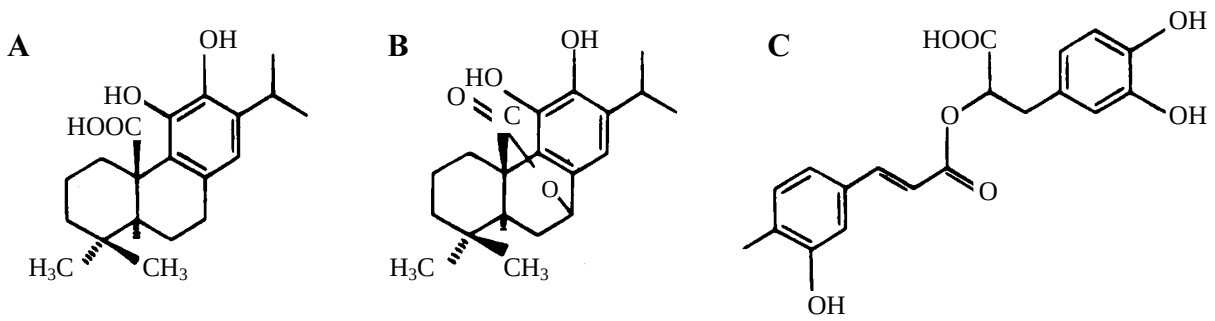

Figure 18: Rosemary component carnosic acid (A), carnosol (B) and rosmarinic acid (C)

In recent years, antioxidant compounds derived from plants, especially phenols, such as carnosol (see fig. 18 B), thymol, eugenol, rosemarinic acid (see fig. 18 C) etc. have become of considerable interest from the viewpoint of dietary antioxidant supplementation and food preservation. Additionally, the consumer prefers these "natural" compounds to the synthetically produced additives, due to their increasing concern over the safety of the latter, including the possible toxicity (VARELTZIS et al., 1997). Many spices have antioxidant properties as they contain flavonoids and phenolic substances. Rosemary (*Rosmarinus officinalis* L.) showed the best antioxidative activity out of a series of plant extracts. Carnosol and carnosic acid (see fig. 18 A) are the most important active components of rosemary extracts. However, it imparts a strong and undesirable taste to products when it is used in high concentrations. But if added in low concentrations they can show a synergistic effect (WADA & FANG, 1992; PALIC et al., 1993; FRANKEL et al., 1996).

For this reason a trial was designed to study the influence of endogenous supplementation of α-tocopherol versus the exogenous addition of rosemary extract with focus on the storage stability of raw fermented sausages of Salami-type. In order to yield even pronounced effects, the risk of oxidation was enhanced by use of frozen stored backfat which resulted from animals which were fed soya oil addition to the feed.

5.2.1 Experimental Design

Fifty-six pigs (Duroc x German Landrace x Piétrain, kindly supplied by Schaumann) were fed a feed consisting of a mixture of grains and soya coarse meal up to live weights of 95 kg. Soya oil had been added in an amount of 2 %, which led to an increase of PUFA in backfat up to more than 20 %. The feed covered the daily requirements of trace elements, amino acids and vitamins. The animals were split up into two groups, the control group receiving the basal diet with about 40 mg tocopherol per kg dry matter, and the second group receiving a supplementation of 200 mg tocopheryl acetate/kg (in a premix by Hoffmann-LaRoche, Basel, Switzerland).

The pigs were raised by Gerd Becker in Bad Bevensen, Germany, and slaughtered at the "Nordfleisch-Zentrale" in Lüneburg, Germany, under normal condition. Meat and backfat were transported to the Department of Meat Technology at the Fachhochschule Lippe (Lemgo, Germany).

5.2.2 Production of Salami

The basic composition consists of 72 % sow meat from the silverside and 28 % cuts of backfat (S IX). Only the backfat was used from the animals of the trial and had been stored in the freezer for 7 months before. An addition of 2.8 % curing salt, 0.5 % sucrose, 0.25 % ground white pepper, 0.05 % sodium ascorbate and 0.025 % starter culture (Duploferment 66®) was mixed into the meat.
Five different batches were produced: three with backfat trimmings from the control group (ca. 40 mg α-tocopherol/kg feed) and two with those from the supplemented group (200 mg α-tocopherol/kg feed). Two different products of rosemary extract were used for this study. The company RAPS & Co. (Kulmbach, Germany) was supplying Stabiloton OS, a powder of rosemary extract, which is yielded by high-pressure extraction with pure carbondioxide. The resulting extract is especially poor in micro-organisms, while the concentration of the interesting components (e.g. carnosic acid ca. 30 %) is high, so that an addition of 0.01-0.03 % is suggested relative to the fat content of the product. The second extract was purchased from the company Chr. Hansen, Denmark. It is also a powder, but the producer stated a lower content of e.g. carnosolic acid (6 %) and recommended an addition of 0.3-0.5 g/kg fat. The rosemary extract was added to the batches according to tab. 9:

Table 9: Addition of Rosemary Extracts to Salami Batches (in %)

	Control #1	Control #2	Control #3	Suppl. #1	Suppl. #2
Stabiloton OS	-	-	0.0084	-	0.0084
Chr. Hansen	-	0.014	-	-	-

After smoking on the third and seventh day, the Salamis were left for ripening (15 °C) for nine days, thereafter sealed in a protective atmosphere of nitrogen and stored at 15 °C for up to 5 months in total.

The production of Salami took place in the Faculty of Food Technology, Department of Meat Technology at the Fachhochschule Lippe (Lemgo, Germany).

Samples were taken immediately after mixing the Salami ingredients (week 0), and Salami sausages periodically after 4, 12 and 21 weeks of storage for determination of α-tocopherol content and fatty acid composition. The samples were stored in the freezer until analysis. α-Tocopherol determinations were made on duplicate 2 g samples (according to the method for meat, refer to appendix 8.1.1), whereas fatty acid compositions (refer to appendix 8.2.1) were calculated from a single 5 g replicate but injected twice.

5.2.3 Results from the Co-operating Institute

When viewing the results of the physical parameters the two supplemented batches showed higher weight loss from the seventh day onwards, but contained more water than the unsupplemented groups. Consequently, water activity was effected by α-tocopherol. The other analyses partly also yielded values which were significantly different, but these were not pronounced enough to draw any conclusion about an influence of either α-tocopherol or rosemary extract. Peroxide numbers did not indicate an association of the two antioxidants with higher stability against oxidation, but TBARS value was highest for the control group without any antioxidants, whereas no distinction could be made between the different antioxidant batches. On the other hand, the "Rancimat-Test" detected a positive influence of added antioxidants, but for α-tocopherol supplementation only in combination with Stabiloton OS. Therefore batch suppl. #2 with α-tocopherol and rosemary extract had the longest persistence against oxidation. Induction periods were mostly, but only slightly longer for the supplemented groups compared to the control groups. Comparing both rosemary extract batches (control #2 and #3) with the control group, the rosemary extracts caused a better stability, with Stabiloton OS showing the higher results compared to rosemary extract by Chr. Hansen. Triangle-tests did not show any significant result; mostly negative results were caused by technological imperfections.

5.2.4 Results and Discussion of α-Tocopherol Contents

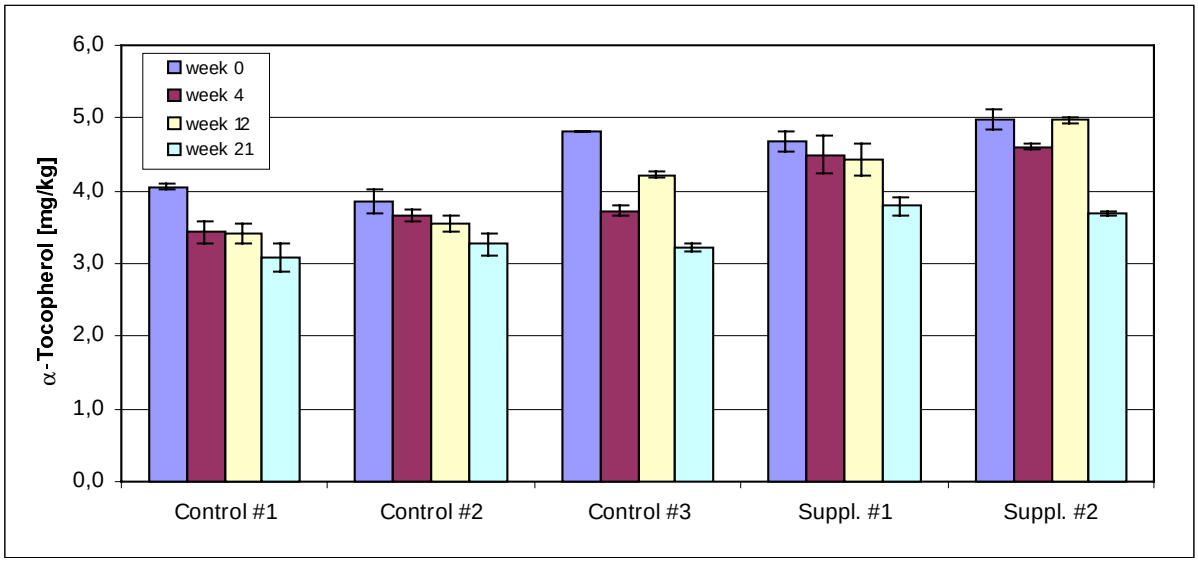

Figure 19: Salami II – alterations of α-tocopherol contents during storage

Salamis were significantly different in α-tocopherol content (see fig. 19, also refer to appendix 8.8.1). The raw mixture of the control batch without rosemary extract contained 4.1 mg α-tocopherol/kg, the corresponding control batches with rosemary extracts contained 3.9 mg/kg (Chr. Hansen) and 4.8 mg/kg (Stabiloton OS), respectively. The batches produced with the

backfat from the supplemented (200 mg tocopherol/kg feed) animals without and with rosemary extract contained 4.7 mg/kg and 5.0 mg/kg, respectively.
A decrease of α-tocopherol content during storage was detected for almost all batches, but the decline was not continual and no correlation could be made up for either α-tocopherol supplementation or addition of rosemary extract. After 21 weeks of storage the control batch without rosemary extract still contained 3.1 mg/kg and the corresponding batches with rosemary extract 3.3 mg/kg and 3.2 mg/kg, respectively. The batches from supplemented animals had α-tocopherol contents of 3.8 mg/kg (without rosemary extract) and 3.7 mg/kg (with rosemary).

A reason, why the groups with addition of Stabiloton OS in the beginning had higher values of α-tocopherol compared to the corresponding groups cannot be given. The extract might have yielded some α-tocopherol, but especially the amount in Control #3 was far too high to be explained by that fact and if this had been the reason, then also group Suppl. #2 should have had a content which is almost 2 mg/kg higher.
The changes during storage pointed out that the concentration of α-tocopherol dropped faster in the control groups compared to supplemented groups and fastest was the reduction in the groups with addition of Stabiloton OS. After 21 weeks of storage the contents within the control groups were about the same, such as the concentrations in the supplemented groups also were almost identical, which means that the effect of rosemary extract addition was down to zero after 5 months of storage. The lowest relative decrease was measured in the group with Hansen´s rosemary extract. It might be concluded from that that if there had been any exogenous α-tocopherol in the rosemary extract it would had oxidised faster than the endogenous and probably also not been regenerated.
Rosemary extracts could improve oxidative stability but were superseded by dietary α-tocopherol supplementation. The rosemary extracts were (maybe) most effective in the beginning by causing higher α-tocopherol contents, but on the long-term endogenous α-tocopherol would be more successful due to its subcellular location. There are no data available on the application of rosemary extract in the production of Salami. But WADA & FANG (1992) have studied the synergistic effect of rosemary extract and α-tocopherol (both added endogenously!) in sardine oil model systems and frozen-crushed fish meat and actually found the rosemary extract to restrain the oxidation of α-tocopherol.

Even after 7 months of storage the level of α-tocopherol in cuts of backfat must have been still higher in the supplemented group compared to the control, as the resulting contents in Salami were higher. Contents in the control group were higher than in the last trial, but the supplemented groups were similar, although the diet in this present trial contained 2 % soya oil. But when comparing with literature results for adipose tissues of pigs after feeding a 200-mg supplemented diet with also an addition of soya oil, it was concluded that at least in the supplemented group a certain reduction had taken place during frozen storage; even when taking into account that only 28 % of this backfat was used according to the recipe.

PFALZGRAF et al. (1995b) had fed pigs with barley and soya bean meal and an additional 3 % soya oil, together with α-tocopheryl acetate at either a basal (20 mg) or a supplemented (200 mg) level. With slaughter at 95 kg, adipose tissue contained 3.1 mg/kg and 12 mg/kg for control and supplemented group, respectively, while MLD contained 2.8 mg/kg and 6.3 mg/kg.
MONAHAN et al. (1992) fed pigs with barley, wheat, soya bean meal and also additional 3 % soya oil with either a basal (10-50 mg/kg) or supplemented (200 mg/kg) level of α-tocopherol to a slaughter weight of 84 kg. In pigs receiving the supplemented diet α-tocopherol concentration was approximately 2.8-times higher in MLD, when compared to pigs fed the basal diet (2.2 compared with 7.0 mg/kg). For adipose tissue the corresponding values were 6.2 and 13 mg/kg. From these results could be seen, that a higher content of α-tocopherol could have been expected for the supplemented Salami batches. It had been shown by MONAHAN et al. (1992), though, that increasing the linoleic acid intake of rats can result in decreased absorption of α-tocopherol. On the contrary, HENGELSBERG et al. (1993) claimed that increasing supply with linoleic acid remains without effect towards uptake of α-tocopherol.

5.2.5 Results and Discussion of Fatty Acid Patterns

Fatty acid patterns in total fat were almost the same for all feeding and production groups and did not change considerably during storage (refer to appendix 8.8.2). Main fatty acids were oleic acid 35 %, palmitic acid 23 %, linoleic acid 15 % and stearic acid 11 %. Compared to the previous trial, oleic acid had decreased about 5 % while linoleic acid had increased with the same percentage. This change was probably due to the influence of soya oil from the diet. Further, linolenic acid was now present with 1.5 instead of 1 %, but all other fatty acids were in the same range of the previous study.
The supplemented groups both showed lower contents of linoleic and linolenic acid than the control groups. The decrease of linoleic acid in total fat during 3 months of storage was about the same in all groups (ca. only 1 % absolute). This value is higher compared to the previous trial where concentrations remained stable, which might be due to the higher absolute content in the Salami. For those batches with rosemary extracts no additional protection against oxidation, resulting in reduced decrease of linoleic and linolenic acid, was detected. On the contrary, WADA & FANG (1992) have studied the synergistic effect of endogenous rosemary extract and α-tocopherol in frozen-crushed fish meat. They actually found the rosemary extract to inhibit the catalytic lipid oxidation of especially PUFA and to lower triglyceride hydrolysis by inactivating the responsible enzyme. Comparably, VARELTZIS et al. (1997) evaluated the effect of adding a natural rosemary extract (Stabiloton WS; 400 mg/kg of total lipids in the mince) to filleted and minced frozen fish. The results showed that the natural antioxidant extract significantly retarded the oxidation process throughout storage. Considerable changes in the percentage of PUFA throughout the period of frozen storage were observed. The percentages of PUFA in the untreated samples were always significantly lower than those of the treated samples, and the oxidation was gradual but slower in the treated samples, which could not be observed in the present trial.

At the beginning, main fatty acids in the phospholipid fraction were linoleic (31 %), palmitic (19 %), stearic (17 %), arachidonic (13 %) and oleic acid (9 %). The most substantial differences to the last trial were that linoleic and arachidonic acid had increased with about 5-6 %, while oleic acid decreased with 9 %. Additional minor changes were increases in palmitic and vaccenic acid, as well as slight decreases in palmitoleic and docosatetraenoic acid.
The alterations during storage were comparable to the previous study. The content of linoleic acid decreased during storage; this time in all groups for about 5 %. Palmitic acid showed a decrease as well, but the extent was also not influenced by supplementation or addition of antioxidant. In general this is also fact for the increases in stearic, oleic and arachidonic acid.
As already described before, such strong variations with even increases in PUFA cannot be attributed to only oxidative processes. Instead, there might be lipolytic changes responsible as well as the analytical method of determination.

From this present trial could be seen that the fatty acid pattern was not influenced by α-tocopherol supplementation of feed or addition of rosemary extract. Consequently, an (additional) protective effect of rosemary extract against oxidation could not be measured. On the contrary, the control group with Hansen´s rosemary extract showed the highest loss of linoleic acid, whereas the control group without any addition of antioxidants showed the lowest.
Together with the results from the Fachhochschule Lippe, which were also partly contradictory and in most cases not in a straight line, it seemed that the long storage time of the cuts from backfat had made it very complicated to study the influences, especially as no batch was prepared with fresh backfat as a real control group. And the presence of the other ingredients that also have certain antioxidant properties had even aggravated the difficulties.
In general it is possible to use frozen-stored backfat for production of meat products, but during processing it has to be taken special care as the susceptibility against oxidation or spoilage is elevated. Enzymes are activated to a high extent, and therefore is the risk of development of rancidity very high for product that are stored for a long time (POLYMENIDIS, 1978).

In evaluation of antioxidants, it is important to distinguish between their effect at different stages of lipid oxidation. Natural antioxidants have been particularly difficult to evaluate because of the complex interfacial phenomena affecting the partition of the antioxidant in multiphase food systems. The actual antioxidant activity of the rosemary compounds in an emulsion system is related to the extent of their interfacial partitioning in the water (FRANKEL et al., 1996). Most natural antioxidants are not very water soluble, which can create problems in meat processing. Pork products, a mixture of lean meat and fat, are probably more suited for water soluble antioxidants since additives are added during the stage in which myofibrillar proteins are extracted and dispersed into the mass (GHIRETTI et al., 1997).

6 SUMMARY

In the present paper, the influence of the natural antioxidant α-tocopherol in feedstuff on the quality of meat (pork and beef) and Salami was evaluated. Dietary α-tocopherol supplementation increases the concentration of α-tocopherol in tissues by "natural" incorporation into the membranes, where it performs its physiological role of protecting membranal lipids and myoglobin from oxidation. As a counterpart to numerous trials, which have been performed under US American conditions, the present studies should instead apply European feeding strategies and should additionally cover alternative sources of feed as well as meat products.
The aims of the different feeding trials were
(i) to measure the extent of incorporation of α-tocopherol from the feed into different tissues, depending on the feed itself, concentration in the feed, as well as duration of feeding,
(ii) to detect modifications of fatty acid composition, influenced by either the feed itself or the concentrations of α-tocopherol in the tissues or other antioxidants present, and
(iii) to study, if the problem of oxidation processes during storage is diminished by the inclusion of α-tocopherol into the tissues.

Pork Experiments. The influence of common feeding techniques (intensive and extensive) as well as contemporary diets (containing lecithin or glycerol; the latter is a waste product from bio-diesel production) on α-tocopherol content was studied for pigs slaughtered at live weights between 100 and 160 kg. For all diets, the basic content was ca. 30 mg α-tocopherol/kg dry matter. Within each feeding group the concentration of α-tocopherol increased with duration, so that the heaviest animals contained the highest levels. There was no difference between intensive and extensive feeding (ca. 2.0-3.0 mg/kg, depending on duration), but there were variations due to addition of lecithin or glycerol. Surprisingly, α-tocopherol values were highest for glycerol (3.2 mg/kg for pigs of 160 kg live weight), and rapeseed oil. The next highest levels were obtained from feeding rapeseed lecithin, but the lowest values (2.2 mg/kg) were obtained from soya lecithin. This might be due to higher contents of polyunsaturated fatty acids in soya lecithin, which oxidise more readily and α-tocopherol content decreases when reacting with oxidative species. Other reasons might be that soya contains rather γ- and δ- than α-tocopherol or that the basic concentrations were actually higher than determined.

Beef Experiments. Bulls were fed either no supplemental α-tocopherol or in a two-factorial supplementation design (quantity x duration). Fatty acid patterns were not significantly affected by α-tocopherol supplementation, and tissue concentrations of α-tocopherol depended more on the daily dose than the duration of supplementation. Concentrations of α-tocopherol in serum and liver reflected the immediate nutritional status of the animal, whereas the α-tocopherol concentrations in adipose and skeletal muscle tissues reflected its long-term nutritional history. α-Tocopherol uptake reached a certain plateau in all tissues, except for liver and adipose tissue. But even the maximum levels in *longissimus* muscle were below the proposed value of 3.0-4.0 mg/kg of fresh muscle as the ideal concentration for prevention of lipid oxidation. Nevertheless, meat from these animals did show enhanced colour stability and resistance to rancidity. Cattle cannot synthesise α-tocopherol and normally obtain it by consuming pasture, whereas grains are relatively poor sources of α-tocopherol, so another study was designed to follow the effect of duration of supplementation with pasture and compare it with silage. In this study, steers were used and α-tocopherol contents were significantly higher the longer the grazing period. In this experiment fatty acid patterns did vary in dependence on feeding, even in the phospholipid fraction that is proposed to be very stable because of its biological role. This was mainly detected in the polyunsaturated fatty acid contents. α-Tocopherol in itself could not be the reason for these effects, so it was concluded, that a synergistic effect with other components in pasture must have been the cause.

Salami Experiments. Salami was used as a minced meat product to study changes during storage. Meat and backfat from pigs supplemented with different amounts of α-tocopherol were mixed with beef and the necessary ingredients (curing salt premix, sugar, ascorbate, smoke). Two different starter cultures were applied, but no difference arose from that. Feed supplementation led to higher α-tocopherol contents, and fatty acid composition in the phospholipid fraction was more stable. The influence of α-tocopherol was also detectable in lower hexanal contents. In a second study, two commercial rosemary extracts were also blended into the Salami mixture. α-Tocopherol concentrations were again correlated with supplementation and with one of the rosemary extracts, but storage stability and changes in fatty acids were not associated. Dietary supplementation of α-tocopherol seemed to be more efficient than *post mortem* addition of rosemary extract. The results from this study though were very contradictory, which was attributed to the experimental cuts of backfat being stored for too long before usage.

6 ZUSAMMENFASSUNG

In der vorliegenden Arbeit wird der Einfluß des natürlichen Antioxidans α-Tocopherol im Futter auf die Qualität von Schweine- und Rindfleisch sowie Salami beschrieben. α-Tocopherolzusatz in der Nahrung erhöht bekanntermaßen die Konzentration im Gewebe, wo es seine physiologische Wirkung entfaltet und Membranlipide und Myoglobin vor Oxidation schützt. Als Gegenstück zu zahlreichen Experimenten, die hauptsächlich unter US-Amerikanischen Fütterungsbedingungen durchgeführt wurden, sollten für die vorliegende Studie Europäische Fütterungsstrategien angewandt und zusätzlich alternative Futterquellen und Fleischprodukte berücksichtigt werden. Die Ziele der verschiedenen Fütterungsversuche waren
(i) das Ausmaß der Einlagerung von α-Tocopherol aus dem Futter in die unterschiedlichen Gewebe, in Abhängigkeit des Futters, der Konzentration im Futter, sowie der Fütterungsdauer zu messen,
(ii) Veränderungen des Fettsäuremusters unter dem Einfluß des Futters selbst oder der Gehalte an α-Tocopherol und anderen Antioxidantien festzustellen, sowie
(iii) zu untersuchen, ob das Problem der Oxidationsprozesse während der Lagerung durch die Einlagerung von α-Tocopherol in die Gewebe verringert wird.

Schweinefleisch Experimente. Untersucht wurden die Einflüsse normaler (intensiv und extensiv) und zeitgemäßer Fütterungstechniken (mit Lecithin oder Glycerin; letzteres als Abfallprodukt der Biodiesel-Produktion) auf den α-Tocopherol Gehalt von Schweinen, geschlachtet bei Lebendgewichten von 100-160 kg. Alle Futter enthielten ca. 30 mg α-Tocopherol pro kg Trockenmasse. Innerhalb der Fütterungsgruppen stieg die α-Tocopherol Konzentration bei längerer Fütterung an, so daß die schwersten Tiere die höchsten Gehalte aufwiesen. Zwischen intensiver und extensiver Fütterung konnten keine Unterschiede festgestellt werden (ca. 2.0-3.0 mg/kg, abhängig von der Fütterungsdauer), aber der Zusatz von Lecithin oder Glycerin bewirkte eine Veränderung. Erstaunlicherweise waren die Werte für Glycerin am höchsten (3.2 mg/kg für Schweine mit 160 kg Lebendgewicht), gefolgt von Rapsöl und Rapslecithin. Die niedrigsten Gehalte (2.2 mg/kg) wurden für Fütterung mit Sojalecithin ermittelt, evtl. aufgrund höherer Gehalte an polyungesättigten Fettsäuren, welche leicht oxidieren und damit indirekt den α-Tocopherolgehalt verringern. Zudem enthält Sojaöl hauptsächlich γ- und δ- statt α-Tocopherol bzw. die Grundgehalte im Futter könnten höher gewesen sein als bestimmt wurde.

Rindfleisch Experimente. Bullen wurden entweder ohne α-Tocopherolzusatz oder mittels zweifaktorieller Supplementierung (Menge x Dauer) aufgezogen. Fettsäuremuster wurden nicht signifikant beeinflußt. Jedoch hatte α-Tocopherol einen starken Einfluß auf die entsprechenden Gewebekonzentrationen; die tägliche Dosis stärker als die Dauer. Die Konzentrationen in Serum und Leber reflektieren dabei den momentanen Ernährungszustand des Tieres, während Fett- und Muskelgewebe die langfristige Versorgung widerspiegeln. Die α-Tocopherolaufnahme erreichte ein bestimmtes Plateau in allen Geweben außer Leber und Fettgewebe. Aber sogar die maximalen Gehalte im *longissimus* Muskel waren unterhalb der vorgeschlagenen 3.0-4.0 mg/kg als idealer Konzentration zur Vermeidung der Lipidoxidation. Trotzdem wies Fleisch dieser Versuchstiere erhöhte Farbstabilität und geringere Neigung zur Fettranzigkeit auf. Rinder können α-Tocopherol nicht synthetisieren und nehmen es normalerweise durch Grünfutter auf, wohingegen Getreide eine relativ schlechte Quelle ist. In einem dritten Versuch wurde daher bei Ochsen der Einfluß der Dauer von Weide- mit Silagefütterung verglichen. α-Tocopherolgehalte waren signifikant höher mit Dauer der Weidefütterung. In dieser Studie veränderten sich auch die Fettsäuremuster in Abhängigkeit der Fütterung, sogar in der Phospholipidfraktion, die aufgrund ihrer biologischen Rolle besonders stabil sein soll. Unterschiede wurden hauptsächlich in den Gehalten polyungesättigter Fettsäuren festgestellt. Da α-Tocopherol nicht der alleinige Grund für diese Einflüsse sein kann, wurde gefolgert, daß ein synergistischer Effekt mit anderen Grünfutters-Komponenten vorliegt.

Salami Experimente. Untersucht wurden Veränderungen während der Lagerung im Fleischprodukt Salami. Fleisch und Speck von Schweinen, die verschiedene Mengen α-Tocopherolzusatz bekommen hatten, wurden mit Rindfleisch und den notwendigsten Zutaten zur Salamiherstellung (Pökelsalz, Zucker, Ascorbat, Rauch) gemischt. Zwei verschiedene Starterkulturen wurden eingesetzt, die zu keinen Unterschieden führten, während der α-Tocopherolzusatz einen höheren Gehalt in der Salami und ein stabileres Fettsäuremuster in der Phospholipidfraktion bewirkte. Auch aus den niedrigeren Hexanalgehalten konnte ein Einfluß des α-Tocopherolzusatzes abgeleitet werden. Im zweiten Versuch wurden zwei kommerzielle Rosmarinextrakte der Salamimischung zugesetzt. Die α-Tocopherolkonzentrationen korrelierten mit dem Futterzusatz und mit einem der Rosmarinextrakte, jedoch nicht mit Lagerstabilität und Veränderungen der Fettsäuremuster. Ein Futterzusatz von α-Tocopherol scheint folglich effektiver zu sein als *post mortem* Zusatz von Rosmarinextrakt. Die Ergebnisse dieses Versuches waren jedoch sehr widersprüchlich, welches den Speckabschnitten zugeschrieben wurde, die bereits zu lange gelagert waren.

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8 APPENDIX

8.1 Determination of α-Tocopherol

8.1.1 Meat Samples and Other Tissues

- Chopping meat or fatty tissue into small pieces
- Weighing meat sample (2.0 g or 1.0 g of fatty tissue) into a brown 50-ml-Schott® bottle
- Addition of 0.5 g of ascorbic acid
- Addition of 5 ml (3 ml for fatty tissues) of methanolic KOH solution (ca. 40 %)
  (375 g KOH, dissolved in 450 ml MeOH, then 750 ml distilled water added)
- Flushing with nitrogen, closing the cap tightly
- Saponification: 40 min, 80 °C (shaking once in a while)
- Addition of 20 ml of 20 % ethanol (EtOH) after cooling
  (for fatty tissues adding 20 ml 50 % EtOH before cooling to the warm solution)
- Extraction of α-tocopherol with 10.0 ml of n-hexane (containing 0.01 % BHT),
  shaking for 10 min at 250 strokes/min, centrifugation at 4000 g
- Injection of 50 µl hexane layer into the SP-HPLC

8.1.2 Serum Samples

- Filling 0.1 g ascorbic acid into a 2-ml-vial
- Addition of 500 µl serum (after thawing and mixing)
- Addition of 500 µl absolute EtOH
- Flushing with nitrogen, closing the cap, shaking vigorously
- Denaturation: 5 min, 80 °C, cooling to room temperature afterwards
- Extraction with 500 µl n-hexane (containing 0.01 % BHT),
  shaking for 5 min at 250 strokes/min, centrifugation at 4000 g
- Injection of 50 µl hexane layer into the SP-HPLC

8.1.3 HPLC-Conditions

Instrument:	Milton Roy Consta Metric 3000 with autosampler LKB Bromma 2157 and cooling system MgW Lauda Kühlbad, type K2RD
Column:	Merck, 250 x 4 mm
Stationary Phase:	LiChrosorb® Si 60, 7 µm
Column temperature:	ambient
Mobile Phase:	n-hexane/ethyl acetate 95:5 (v:v), isocratic, recycle
Flow / Pressure:	1 ml/min, approximately 30 bar
Detection:	Merck-Hitachi, F-1050 Fluorescence Spectrophotometer, Excitation λ = 285 nm, Emission λ = 325 nm
Integrator:	Merck-Hitachi D-2500 Chromato-Integrator
Injection volume:	50 µl
Standards:	0.1 - 2.5 µg α-tocopherol/ml n-hexane (containing 0.01 % BHT)
Run time:	12 min
Calculation:	External Standard Method, peak area

8.2 Determination of Fatty Acid Patterns

8.2.1 Meat and Liver Samples

- Chopping meat or liver into small pieces
- Weighing meat sample (5 g muscle or 2 g liver) into 100-ml centrifugation glass
- First extraction with 15 ml dichloromethane (DCM)/MeOH (4:1, v:v) using ultra-turrax, two more extractions after dispersing with 10 ml DCM/MeOH using sonification, a fourth extraction after dispersing with 10 ml n-hexane using sonification
- Evaporating combined extracts, resolving in 12.5 ml DCM (saturated with water)

⇒ yielding the "DCM extract"

Total Fatty Acid Pattern in Meat and Liver Samples

- Using a 0.5 ml aliquot of the DCM extract
- Evaporating DCM, resolving in 1 ml n-hexane
- Addition of 0.2 ml methanolic KOH solution (11 %), shaking manually, neutralisation with 2 N HCl after 10 min, addition of 2 ml n-hexane (containing 0.01 % BHT), shaking manually
- Injection of 3 µl hexane layer into the GC

Phospholipid Fatty Acid Pattern in Meat and Liver Samples

- Conditioning a silica-SPE column with 5 ml n-hexane and 8 ml DCM (saturated with water)
- Placing 3.0 ml (2.0 ml for liver tissue) DCM extract onto the column
- Washing with 2 ml DCM (saturated with water)
- Eluting neutral lipids with 10 ml diethylether/n-hexane (4:1, v:v) (not used furthermore)
- Elution of phospholipids with 30 ml MeOH/water (98:2, v:v) into 50-ml-Schott® bottles
- Addition of 1.2 ml methanolic KOH solution (11 %) to MeOH extract, shaking manually and neutralisation with 2 N HCl after 10 min
- Addition of 20 ml distilled water
- Extraction with 1.0 ml n-hexane (containing 0.01 % BHT), shaking for 10 min at 250 strokes/min, centrifugation at 4000 g
- Injection of 2 µl hexane layer into the GC

8.2.2 Kidney Fat

- Chopping fat tissue into small pieces
- Rendering (laboratory scale) the fat in a beaker, 30 min at 80 °C
- Diluting 200 mg rendered fat with 3 ml n-hexane (containing 0.01 % BHT) in a 10-ml Pyrex®
- Addition of 0.2 ml methanolic KOH solution (11 %), shaking manually and neutralisation with 2 N HCl after 10 min
- Placing 100 µl of hexane layer in a vial, diluting by adding 1 ml n-hexane
- Injection of 1 µl hexane solution into the GC

8.2.3 Serum Samples

- Pipetting 1.0 ml of blood serum into a 10-ml bottle
- Threefold extraction with 3 ml DCM/MeOH (2:1, v:v), shaking manually, centrifugation
- Extracts are dried with sodium sulphate and collected
- Addition of 200 µl BHT solution (1 % in MeOH), evaporating with nitrogen stream and resolving in 1.0 ml of n-hexane
- Conditioning of an amino-SPE column with 6 ml n-hexane
- Placing extract (1.0 ml) onto the column
- Elution of cholesterol esters with 8 ml n-hexane/DCM (9:1, v:v) into 10-ml Pyrex®
- Elution of triglycerides with 4 ml diethylether/n-hexane (1:1, v:v) into 10-ml Pyrex®
- Elution of phospholipids with 6 ml MeOH into 10-ml Pyrex®
- Evaporating extracts under nitrogen stream

Derivatisation of Triglyceride and Phospholipid Fractions:

- Resolving the dry extract with 1 ml n-hexane
- Addition of 0.5 ml methanolic KOH solution (11 %), shaking manually and neutralisation with 2 N HCl after 10 min
- Pipetting hexane layer into a vial, evaporating the solvent with a nitrogen stream and resolving with 200 µl n-hexane (containing BHT)
- Injection of 5 µl hexane solution into the GC

Derivatisation of Cholesterol Ester Fraction:

- Resolving the dry extract with 2 ml MeOH and 1 ml toluene
- Addition of 0.5 ml acetyl chloride (caution: getting hot and foaming!) shaking manually, heating for 1 h at 100 °C, cooling to room temperature
- Neutralisation with sodium carbonate (5 %) (caution: CO_2 development!)
- Pipetting organic layer into a vial, evaporating the solvent with a nitrogen stream and resolving with 200 µl n-hexane (containing BHT)
- Injection of 5 µl hexane solution into the GC

8.2.4 GC-Conditions

Instrument:	Hewlett Packard HP 5890 and HP 6890, respectively with autosampler and software HP Chem Station
Injector:	200 °C
Column:	SGE and Chrompack, 50 m, 0.25 mm ID, 0.25 µm phase
Stationary Phases:	SGE BPX 70 (70 % Cyanopropylpolysilphenylensiloxyn) and Chrompack CP-Sil 88 (100 % cyanopropyl), respectively
Flow:	1 ml Helium/min
Split:	1:10
Temperatures:	Programme I: 150 °C, 4 min isothermal with 2 °C/min up to 180 °C, 8 min isothermal with 2 °C/min up to 206 °C, 5 min isothermal with 2 °C/min up to 250 °C, 5 min isothermal

	Programme II: 150 °C, 4 min isothermal
	with 1 °C/min up to 180 °C, 5 min isothermal
	with 2 °C/min up to 206 °C, 0 min isothermal
	with 10 °C/min up to 235 °C, 5 min isothermal
Detector:	FID, 260 °C
	300 ml oxygen/min, 40 ml hydrogen/min
Make-up:	50 ml nitrogen/min
Standards:	various FAMEs in n-hexane (containing 0.01 % BHT), see 8.4
Run times:	72 min and 60 min, respectively
Calculation:	External Standard Method,
	peak area, mostly corrected by response factors

8.3 Analysis of Hexanal and Other Volatile Aldehydes

- Conditioning the Tenax adsorption tube in the GC
- Preheating the waterbath to 40 °C
- Flushing the closed apparatus 5 min with nitrogen
- Homogenising meat (pre-cut into small pieces)
- Placing 3.0 g meat sample in the glass extraction flask of the apparatus
- Adding 3.0 µl of the Internal Standard (131.1 mg trans-2-hexenal/25 ml EtOH)
- Setting the piston into position, closing the apparatus
- Switching on the piston (stirring)
- Extraction: 40 min, nitrogen flow 40 ml/min, vacuum 700 mbar
- Screwing Tenax adsorption tube into the CHIS for thermal desorption (2 min)
- Starting GC analysis after desorption time of 2 min

GC-Conditions

Instrument:	Carlo Erba 4300 Strumentazione
Injector:	CHIS, 290 °C
Column:	J&W Scientific, 30 m, 0.32 mm ID, 0.25 µm phase
Stationary Phase:	DB-5 (Methylpolysiloxane + 5 % Phenylpolysiloxane)
Flow:	1.1 ml Helium/min (DESORB), 1.4 ml/min (RUN)
Temperature:	35 °C, 3 min isothermal (including DESORB)
	with 5 °C/min up to 200 °C, 2 min isothermal
Detector:	FID, 290 °C
	300 ml oxygen/min, 20 ml hydrogen/min
Make-up:	30 ml nitrogen/min
Integrator:	Merck-Hitachi D-2000 Chromato-Integrator
Standards:	various aldehydes in n-hexane (containing 0.01 % BHT), see 8.4
Run time:	38 min
Calculation:	Internal Standard Method, peak areas

8.4 Standards, Chemicals and Instruments

Fatty Acid Methyl Ester Standard (Table A-1)

	systematic name		concentration [mg/20 ml hexane]
C10	decanoic acid	capric acid	28.8
C12	dodecanoic acid	lauric acid	31.2
C13	tridecanoic acid		31.2
C14	tetradecanoic acid	myristic acid	32.0
C14:1 tr9	9-trans tetradecenoic acid	myristelaidic acid	
C14:1 c9	9-cis tetradecenoic acid	myristoleic acid	32.5
C15	pentadecanoic acid		32.2
C15:1 c9	9-cis pentadecenoic acid		
C16	hexadecanoic acid	palmitic acid	25.8
C16:1 tr9	9-trans hexadecenoic acid	palmitelaidic acid	
C16:1 c9	9-cis hexadecenoic acid	palmitoleic acid	35.1
C17	heptadecanoic acid	margaric acid	27.8
C17:1 c9	9-cis heptadecenoic acid		
C18	octadecanoic acid	stearic acid	28.2
C18:1 tr9	9-trans-octadecenoic acid	elaidic acid	
C18:1 c9	9-cis-octadecenoic acid	oleic acid	35.0
C18:1 c11	11-cis-octadecenoic acid	vaccenic acid	27.2
C18:2 c9	all-cis 9,12 octadecadienoic acid	linoleic acid	31.7
C18:3 c6	all-cis 6,9,12 octadecatrienoic acid	γ-linolenic acid	18.4
C18:3 c9	all-cis 9,12,15 octadecatrienoic acid	linolenic acid	34.6
C18:4 c6	all-cis 6,9,12,15 octadecatetraenoic acid		30.0
C20	eicosanoic acid	arachinic acid	11.3
C20:1 c11	11-cis eicosenoic acid		19.4
C20:2 c11	all-cis 11,14 eicosadienoic acid		30.8
C20:3 c8	all-cis 8,11,14 eicosatrienoic acid		30.8
C20:3 c11	all-cis 11,14,17 eicosatrienoic acid		13.9
C20:4 c5	all-cis 5,8,11,14 eicosatetraenoic acid	arachidonic acid	42.7
C20:5 c5	all-cis 5,8,11,14,17 eicosapentaenoic acid		6.4
C20	eicosanoic acid	arachinic acid	11.3
C20:1 c11	11-cis eicosenoic acid		19.4
C20:2 c11	all-cis 11,14 eicosadienoic acid		30.8
C20:3 c8	all-cis 8,11,14 eicosatrienoic acid		30.8
C20:3 c11	all-cis 11,14,17 eicosatrienoic acid		13.9
C20:4 c5	all-cis 5,8,11,14 eicosatetraenoic acid	arachidonic acid	42.7
C20:5 c5	all-cis 5,8,11,14,17 eicosapentaenoic acid		6.4
C21	heneicosanoic acid		32.8
C22	docosanoic acid	behenic acid	28.3
C22:1 c13	13-cis docosenoic acid	erucic acid	36.1
C22:2 c13	all-cis 13,16 docosadienoic acid		19.3
C22:3 c13	all-cis 13,16,19 docosatrienoic acid		14.9
C22:4 c7	all-cis 7,10,13,16 docosatetraenoic acid		20.7
C22:5 c7	all-cis 7,10,13,16,19 docosapentaenoic acid		
C22:6 c4	all-cis 4,7,10,13,16,19 docosahexaenoic acid		7.7
C24	Tetracosanoic acid	lignoceric acid	27.8
C24:1 c15	15-cis tetracosenoic acid	nervonic acid	25.5

Chemical Substances (Table A-2)

chemicals	Company	molar mass M [g/mol]	Hazard symbols	R (risk phrases)	S (safety phrases)	MAK [ml/m^3]
DL-α-tocopherol	Sigma	430.72				
acetyl chloride	Merck	78.50	F, C	11 - 14 - 34	9 - 16 - 26 - 45	
ascorbic acid	Merck	176.13				
BHT	Merck	220.36				
decanal	Sigma	156.27			26 - 36	
dichloromethane	Merck	84.93	Xn	40	23 - 24/25 - 36/37	100
diethyl ether	Merck	74.12	F+	12 - 19	9 - 16 - 29 - 33	400
ethanol	Merck	46.07	F	11	7 - 16	1000
ethyl acetate	Merck	88.10	F	11	16 - 23 - 29 - 33	400
heptanal	Sigma	114.19		10		
hexanal	Sigma	100.16		10		
n-hexane	Merck	86.18	F, Xn	11 - 20 - 48	9 - 16 - 24/25 - 29 - 51	50
hydrochloric acid	Merck	36.46	C	34 - 37	26 – 36/37/39 - 45	5
methanol	Merck	32.04	T, F	11 - 23/25	7 - 16 - 24 - 45	200
nonanal	Sigma	142.24	Xi	38		
octanal	Sigma	128.22		10		
pentanal	Sigma	86.13	F, Xi	11 - 36/37/38		
potassium hydroxide	Merck	56.11	C	35	26 - 37/39 - 45	
sodium carbonate	Merck	105.99	Xi	36	22 - 26	
sodium sulfate	Merck	142.04				
t-2-hexenal	Sigma	98.15				
t-2-nonenal	Sigma	140.23				
t-2-octenal	Sigma	126.20				
toluene	Merck	92.14	F, Xn	11 - 20	16 - 25 - 29 - 33	50
undecanal	Sigma	170.30				
FAMEs	Sigma					

Instruments

Balance:	Sartorius
Silica-SPE-columns:	Supelco, Supelclean LC - Si, 500 mg, 3 ml
Amino-SPE-columns:	Alltech, Extract-Clean NH_2, 500 mg, 2.8 ml
Rotation evaporator:	Heidolph VV 2000 and WB 2000
Shaking machine:	Edmund Bühler B5
Drying oven:	Heraeus BI 3645 E
Ultra-Turrax:	Janke & Kunkel T 25, IKA Labortechnik
Sonificator:	Bransonic 12
Centrifuge:	Heraeus Christ Labofuge 6000

8.5 Statistics

The statistic analyses have been carried out according to following formulas:

8.5.1 Standard Deviation

$$s = \sqrt{\frac{\sum (x_i - x)^2}{n-1}}$$

with s = standard deviation
x_i = single value
x = mean value
n = number of samples

$$s_t = \sqrt{s_1^2 + s_2^2 + \ldots + s_n^2}$$

with s_t = total deviation
s_1, s_2, ..., s_n = single deviations of samples 1, 2, ..., n

8.5.2 Analysis of Significance

$$p = \frac{x_a - x_b}{s / \sqrt{n}}$$

with p = probability
x_a, x_b = mean value of samples to be compared
s = standard deviation
n = number of samples

$p < 0.01$	highly significant
$0.01 < p < 0.05$	significant
$p > 0.05$	not significant

8.5.3 Multivariate Analysis

Beef data were analysed by *The Unscrambler´*s (version 7.5 alfa 1, CAMO, Norway) principal least squares regression (PLSR2) with full cross validation and showing loading plots or scores plots (2-dimensional scatter plots of variables and samples, respectively) as results. The measured variables were used as X-data and the design variables as Y-data and all were weighed by 1/standard deviation.

8.6 Pork Experiments

8.6.1 Results of Tocopherol Determinations

Table A-3: Pork I – Mean and Standard Deviation of α-Tocopherol in Muscle

slaughter / feeding	100 kg		120 kg		140 kg		160 kg	
	mean	deviation	mean	deviation	mean	deviation	mean	deviation
intensive	2.07 [a]	0.28	1.90 [a]	0.35	2.79 [b]	0.24	3.24 [c]	0.32
extensive	2.18 [a]	0.33	2.17 [a]	0.29	2.63 [b]	0.24	3.09 [c]	0.30

Mean values with different superscripts in a row are significantly different ($p < 0.05$).

Table A-4: Pork II - Mean and Standard Deviation of α-Tocopherol in Muscle

slaughter / feeding	100 kg		130 kg		160 kg	
	mean	deviation	mean	deviation	mean	deviation
rapeseed oil	2.60 $^{a}_{A}$	0.23	3.04 $^{b}_{A}$	0.10	3.04 $^{a,b}_{A}$	0.70
rapeseed lecithin	2.33 $^{a}_{A, B}$	0.27	2.95 $^{b}_{A}$	0.48	2.84 $^{b}_{A}$	0.15
soya lecithin	2.14 $^{a}_{B,C}$	0.38	2.21 $^{a}_{B}$	0.39	2.17 $^{a}_{B}$	0.20
rapeseed glycerine	2.63 $^{a}_{A,C}$	0.64	2.97 $^{a}_{A}$	0.31	3.18 $^{a}_{A}$	0.44

Mean values with different superscripts in a row are significantly different ($p < 0.05$).
Mean values with different subscripts in a column are significantly different ($p < 0.05$).

8.7 Beef Experiments

8.7.1 Results of Tocopherol Determinations

Table A-5: Young Bulls I - Mean and Standard Deviation in Tissues and Serum

	control group C 80		control group C 120		feeding group A 80	
	mean	deviation	mean	deviation	mean	deviation
MLD	0.42 [a]	0.17	0.69 [b]	0.16	1.11 [c]	0.33
MST	0.53 [a]	0.13	0.76 [b]	0.19	1.48 [c]	0.23
serum	0.86 [a,b]	0.24	0.96 [a]	0.28	1.38 [a,b,c]	0.46
kidney fat	1.89 [a]	0.38	2.29 [b]	0.63	3.91 [c]	0.70
liver	2.57 [a]	0.50	2.97 [a]	1.03	5.88 [b]	0.65

	feeding group A 120		feeding group B 80		feeding group B 120	
	mean	deviation	mean	deviation	mean	deviation
MLD	1.30 [c]	0.25	1.52 [d]	0.26	2.79 [e]	0.63
MST	1.91 [d]	0.52	2.90 [e]	0.53	3.56 [e]	1.17
serum	1.75 [c]	0.51	3.09 [d]	0.58	3.86 [e]	0.66
kidney fat	4.24 [c]	0.49	6.87 [d]	0.53	8.41 [e]	1.26
liver	5.78 [b]	1.15	12.71 [c]	0.77	16.82 [d]	1.97

Mean values with different superscripts in a row are significantly ($p < 0.05$) different.

Table A-6: Young Bulls II - Mean and Standard Deviation in Tissues and Serum

	control group C		feeding group D 180		feeding group D 270	
	mean	deviation	mean	deviation	mean	deviation
MLD	0.42 [a]	0.11	2.40 [b]	0.19	2.86 [c]	0.37
MST	0.33 [a]	0.11	1.85 [b]	0.28	2.60 [c]	0.39
serum	0.35 [a]	0.08	1.67 [b]	0.37	2.25 [c]	0.39
kidney fat	1.25 [a]	0.36	5.43 [b]	1.05	7.19 [c]	0.95
liver	0.99 [a]	0.21	6.45 [b]	0.95	8.05 [c]	1.33

Mean values with different superscripts in a row are highly significant ($p < 0.01$) different.

Table A-7: Steers - Mean and Standard Deviation in Tissues and Serum

	group Silage		group Mixed		group Pasture	
	mean	deviation	mean	deviation	mean	deviation
MLD	0.94 [a]	0.16	2.29 [b]	0.46	3.57 [c]	0.57
serum	0.90 [a]	0.50	1.75 [b]	0.47	5.37 [c]	1.58
kidney fat	2.26 [a]	0.48	5.41 [b]	1.11	10.34 [c]	2.86
liver	2.98 [a]	1.11	4.95 [b]	2.19	13.16 [c]	2.70

Mean values with different superscripts in a row are highly significant ($p < 0.01$) different.
(except liver S:M - only significant $p < 0.05$)

8.7.2 Results of Fatty Acid Determinations

Table A-8: Young Bulls I – Mean and Standard Deviation of Total Fat in Muscles

Table A-9: Young Bulls I – Mean and Standard Deviation of Total Fat in Liver and Kidney

Table A-10: Young Bulls I – Mean and Standard Deviation of Phospholipids in Muscles

Table A-11: Young Bulls I – Mean and Standard Deviation of Phospholipids in Liver

Table A-12: Young Bulls I – Mean and Standard Deviation in Serum Fractions I

Table A-13: Young Bulls I – Mean and Standard Deviation in Serum Fractions II

Table A-14 : Young Bulls II - Mean and Standard Deviation of Total Fat in Tissues

Table A-15 : Young Bulls II - Mean and Standard Deviation of Phospholipids in Tissues

Table A-16 : Young Bulls II - Mean and Standard Deviation in Serum Fractions

Table A-17 : Steers - Mean and Standard Deviation of Total Fat in Tissues

Table A-18 : Steers - Mean and Standard Deviation of Phospholipids in Tissues

Table A-19 : Steers - Mean and Standard Deviation in Serum Fractions

Table A-8: Young Bulls I – Mean and Standard Deviation of Total Fat in Muscles

	MLD												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	1,67	0,43	1,87	0,58	1,89	0,36	2,13	0,38	1,14	0,75	2,20	0,45	1,82
C 14:1 c9	0,43	0,35	0,05	0,08	0,11	0,19	0,30	0,18	n.d.	n.d.	0,11	0,19	0,20
C 15	0,28	0,34	0,09	0,15	n.d.	n.d.	0,34	0,08	n.d.	n.d.	0,07	0,13	0,20
C 16	24,61	2,10	24,78	2,94	26,69	0,63	23,81	1,58	23,27	1,16	26,05	1,29	24,87
C 16:1 tr.	n.d.	n.d.	0,04	0,07	n.d.	n.d.	0,04	0,07	n.d.	n.d.	n.d.	n.d.	0,04
C 16:1 c9	2,57	0,68	2,51	1,07	2,47	0,17	2,06	0,32	1,60	1,01	2,33	0,38	2,26
C 17	0,83	0,54	0,68	0,39	0,34	0,39	1,04	0,11	0,42	0,42	0,74	0,06	0,68
C 17:1 tr.	0,60	0,42	0,54	0,32	0,59	0,38	0,56	0,05	n.d.	n.d.	0,75	0,25	0,61
C 17:1	n.d.	n.d.	0,06	0,10	0,31	0,31	0,07	0,12	n.d.	n.d.	0,18	0,31	0,16
C 18	15,81	1,47	16,60	2,74	15,39	1,65	18,15	1,70	15,84	0,55	16,80	0,32	16,43
C 18:1 tr.	1,28	0,55	1,33	0,31	1,88	0,93	1,33	0,38	0,87	0,17	0,79	0,12	1,25
C 18:1 c9	31,11	4,66	34,96	5,73	35,90	2,34	35,25	4,25	32,38	2,07	34,32	2,02	33,99
C 18:1 c11	2,09	0,49	1,89	0,31	2,32	0,50	1,57	0,12	1,51	0,88	2,29	0,64	1,95
C 18:2	9,34	2,38	9,75	6,73	9,01	1,70	7,60	2,51	15,34	1,82	9,08	0,46	10,02
C 18:3 c9	0,58	0,45	0,07	0,11	n.d.	n.d.	0,27	0,16	n.d.	n.d.	n.d.	n.d.	0,31
C 20:3 c8	1,34	0,86	0,64	0,57	0,66	0,40	0,50	0,39	3,37	3,50	0,72	0,06	1,21
C 20:4	2,78	1,38	3,24	2,33	3,23	0,83	2,07	1,06	4,09	2,37	2,99	0,21	3,07
C 22:4	0,66	0,80	0,04	0,06	n.d.	n.d.	0,18	0,16	0,16	0,27	0,16	0,16	0,24
∑ SFA	43,20		44,02		44,31		45,47		40,67		45,86		43,92
∑ MUFA	38,08		41,38		43,58		41,18		36,36		40,77		40,23
∑ PUFA	14,70		13,74		12,90		10,62		22,96		12,95		14,65

	MST												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	1,23	0,10	1,65	0,41	1,57	0,28	0,46	0,80	0,73	0,45	1,70	0,11	1,22
C 14:1 c9	n.d.	n.d.	0,17	0,33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,17
C 15	n.d.	n.d.	0,06	0,13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,06
C 16	22,00	1,32	23,41	2,90	24,32	1,29	25,55	3,94	20,98	1,66	24,49	0,91	23,46
C 16:1 c9	1,78	0,27	2,64	0,66	2,35	0,44	1,41	0,98	1,26	0,75	2,51	0,51	1,99
C 17	0,57	0,33	0,40	0,50	0,13	0,26	n.d.	n.d.	0,43	0,43	0,38	0,38	0,38
C 17:1 tr.	0,30	0,30	0,35	0,43	0,33	0,40	0,31	0,53	0,37	0,37	0,20	0,35	0,31
C 17:1	n.d.	n.d.	n.d.	n.d.	0,13	0,26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,13
C 18	16,44	1,65	15,14	1,84	15,71	1,91	13,39	2,38	15,53	1,34	15,07	2,07	15,21
C 18:1 tr.	1,55	0,50	1,21	0,38	1,20	0,24	1,49	0,41	0,98	0,07	1,19	0,21	1,27
C 18:1 c9	35,83	0,68	34,75	6,82	38,16	2,50	27,93	4,90	31,81	2,58	34,99	2,89	33,91
C 18:1 c11	2,50	0,64	1,63	0,87	2,37	0,35	1,09	1,10	1,55	0,91	1,98	0,24	1,85
C 18:2	12,12	1,11	10,61	4,25	9,27	2,21	16,28	3,18	16,59	3,61	11,01	2,35	12,65
C 20:3 c8	0,78	0,46	0,39	0,48	0,56	0,53	n.d.	n.d.	1,45	0,39	0,58	0,58	0,75
C 20:4	4,70	0,56	4,11	1,84	3,63	0,93	7,96	2,04	6,89	1,34	4,01	1,08	5,22
C 22:4	0,62	0,12	0,11	0,21	0,28	0,37	n.d.	n.d.	0,41	0,41	0,35	0,35	0,35
∑ SFA	40,24		40,66		41,73		39,40		37,67		41,64		40,22
∑ MUFA	41,96		40,75		44,54		32,23		35,97		40,87		39,64
∑ PUFA	18,22		15,22		13,74		24,24		25,34		15,95		18,79

all values expressed as percentage of total fatty acids

Table A-9: Young Bulls I – Mean and Standard Deviation of Total Fat in Liver and Kidney

	Liver												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	0,39	0,39	n.d.	n.d.	0,35	0,36	0,36	0,36	0,34	0,42	0,36	0,36	0,36
C 14:1 c9	n.d.	n.d.	n.d.	n.d.	0,10	0,17	0,07	0,11	n.d.	n.d.	n.d.	n.d.	0,09
C 15	n.d.	n.n	n.d.	n.d.	0,74	0,78	0,16	0,28	0,76	0,57	0,87	0,61	0,63
C 16	8,18	0,51	9,97	1,06	10,11	0,66	9,16	1,20	8,88	0,62	7,90	0,81	9,03
C 16:1 tr.	0,27	0,27	n.d.	n.d.	0,26	0,28	n.d.	n.d.	0,16	0,28	0,55	0,45	0,31
C 16:1 c9	0,34	0,20	0,14	0,25	0,64	0,46	0,58	0,17	0,55	0,09	0,77	0,33	0,50
C 17	1,08	0,14	0,71	0,42	0,99	0,07	0,87	0,20	1,06	0,13	1,14	0,18	0,98
C 17:1 tr.	0,39	0,39	n.d.	n.d.	0,51	0,54	0,56	0,56	0,31	0,53	0,48	0,54	0,45
C 17:1	0,58	0,58	n.d.	n.d.	0,52	0,54	0,26	0,27	0,57	0,49	0,5	0,51	0,49
C 18	33,75	2,16	34,90	0,96	30,50	1,81	33,16	1,27	33,12	2,27	32,37	2,21	32,97
C 18:1 tr.	0,78	0,06	0,91	0,22	0,71	0,15	0,73	0,09	0,76	0,13	0,97	0,12	0,81
C 18:1 c9	11,02	1,30	12,91	0,85	12,89	2,17	11,59	2,47	12,24	0,43	9,13	1,45	11,63
C 18:1 c11	0,99	0,12	0,87	0,52	1,09	0,19	1,08	0,08	1,02	0,12	0,92	0,17	1,00
C 18:2	14,77	2,37	12,98	1,13	14,38	2,03	13,19	0,64	15,66	0,67	14,02	2,45	14,17
C 18:3 c6	n.d.	n.d.	n.d.	n.d.	0,13	0,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,13
C 18:3 c9	0,33	0,33	n.d.	n.d.	0,57	0,25	0,45	0,13	0,85	0,19	0,73	0,29	0,59
C 20:2	n.d.	n.d.	n.d.	n.d.	0,08	0,13	n.d.	n.d.	0,12	0,20	n.d.	n.d.	0,10
C 20:3 c8	4,72	0,60	6,47	1,05	4,23	1,13	6,07	0,89	4,19	0,94	4,27	0,20	4,99
C 20:4	12,34	1,34	13,32	0,63	11,75	0,92	12,06	1,10	11,13	1,74	11,87	0,73	12,08
C 20:3 c11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,35	0,61	0,35
C 22:4	2,06	0,24	2,15	0,12	1,78	0,55	1,77	0,34	1,70	0,21	1,94	0,64	1,90
C 22:6	1,00	0,20	1,12	0,04	0,83	0,08	1,16	0,24	0,86	0,17	0,93	0,22	0,98
∑ SFA	43,40		45,58		42,69		43,71		44,16		42,64		43,70
∑ MUFA	14,37		14,83		16,72		14,87		15,61		13,32		14,95
∑ PUFA	35,22		36,04		33,75		34,70		34,51		34,11		34,72

	Kidney												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	3,44	0,40	3,13	0,35	2,71	0,46	3,04	0,24	3,14	0,22	2,87	0,71	3,06
C 14:1 c9	0,37	0,09	0,47	0,05	0,38	0,13	0,46	0,05	0,49	0,10	0,35	0,06	0,42
C 15	0,46	0,06	0,48	0,07	0,36	0,13	0,40	0,19	0,46	0,07	0,31	0,06	0,41
C 16	26,38	2,29	25,51	1,11	23,39	3,26	25,02	0,75	26,41	1,13	24,31	2,95	25,17
C 16:1 c9	1,28	0,19	1,37	0,35	1,02	0,54	1,36	0,24	1,35	0,24	1,30	0,20	1,28
C 17	1,36	0,14	1,41	0,13	1,37	0,05	1,27	0,13	1,37	0,09	1,31	0,12	1,35
C 17:1 tr.	0,31	0,07	0,38	0,10	0,34	0,08	0,33	0,13	0,35	0,06	0,40	0,04	0,35
C 17:1	0,17	0,05	0,19	0,12	0,22	0,04	0,19	0,04	0,15	0,09	0,2	0,11	0,19
C 18	31,31	3,35	30,82	4,52	34,26	6,85	30,36	2,31	30,24	2,10	29,71	1,82	31,12
C 18:1 tr.	1,91	0,39	2,05	0,20	1,70	0,55	1,88	0,45	1,60	0,09	2,01	0,44	1,86
C 18:1 c9	26,42	1,30	27,47	3,29	27,82	2,77	29,46	4,68	27,47	1,30	30,73	1,27	28,23
C 18:1 c11	1,02	0,10	1,06	0,16	1,00	0,13	1,08	0,12	1,00	0,02	1,13	0,13	1,05
C 18:2	2,67	0,35	2,50	0,37	2,22	0,35	2,59	0,52	2,69	0,24	2,63	0,32	2,55
C 18:3 c9	0,14	0,08	0,19	0,06	0,13	0,09	0,17	0,07	0,15	0,04	0,14	0,05	0,15
∑ SFA	62,95		61,35		62,09		60,09		61,62		58,51		61,10
∑ MUFA	31,48		32,99		32,48		34,76		32,41		36,12		33,37
∑ PUFA	2,81		2,69		2,35		2,76		2,84		2,77		2,70

all values expressed as percentage of total fatty acids

Table A-10: Young Bulls I – Mean and Standard Deviation of Phospholipids in Muscles

	MLD												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	0,21	0,11	0,15	0,02	0,14	n.d.	0,11	0,07	0,07	0,07	0,14	0,01	0,14
C 14:1 c9	0,03	0,05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,03	0,04	n.d.	n.d.	0,03
C 15	0,19	0,04	0,17	0,01	0,16	0,01	0,16	0,01	0,16	0,02	0,15	0,02	0,17
C 16	20,68	0,73	20,99	2,65	21,42	0,36	21,43	0,53	19,59	2,57	20,16	1,72	20,71
C 16:1 tr.	0,28	0,16	0,17	0,04	0,23	0,06	0,18	0,02	0,22	0,03	0,15	n.d.	0,21
C 16:1 c9	0,94	0,28	0,77	0,12	1,06	0,20	0,68	0,06	0,86	0,21	0,82	0,14	0,86
C 17	0,67	0,17	0,54	0,03	0,55	0,03	0,56	0,08	0,55	0,06	0,53	0,10	0,57
C 17:1 tr.	0,61	0,06	0,58	0,05	0,66	0,08	0,51	0,07	0,67	0,11	0,51	0,12	0,59
C 17:1	1,13	0,14	0,93	0,22	1,06	0,12	0,88	0,13	0,78	0,46	0,82	0,29	0,93
C 18	17,16	1,09	16,53	1,41	16,55	1,03	17,11	1,16	15,59	2,07	16,56	0,47	16,58
C 18:1 tr.	0,36	0,04	0,35	0,05	0,33	0,10	0,33	0,07	0,42	0,19	0,32	0,03	0,35
C 18:1 c9	17,25	1,51	18,74	2,30	21,23	3,30	16,53	1,74	19,76	3,54	17,85	1,47	18,56
C 18:1 c11	2,82	0,35	2,84	0,65	2,85	0,39	2,68	0,46	2,70	0,23	2,86	0,47	2,79
C 18:2	20,84	1,34	20,47	2,87	18,87	1,24	22,24	0,94	21,45	1,34	22,19	1,87	21,01
C 18:3 c6	0,13	0,07	0,13	0,13	0,10	0,10	0,06	0,10	0,16	0,09	0,10	0,10	0,11
C 18:3 c9	0,75	0,08	0,74	0,09	0,68	0,08	0,72	0,06	0,73	0,13	0,75	0,09	0,73
C 20:2	0,18	0,01	0,20	0,02	0,13	0,07	0,22	0,02	0,16	0,09	0,22	0,03	0,19
C 20:3 c8	1,85	0,25	1,98	0,17	1,69	0,29	1,86	0,25	2,02	0,27	2,00	0,21	1,90
C 20:4	7,93	0,65	8,36	0,29	7,46	0,38	8,53	0,14	8,73	0,75	8,57	0,43	8,26
C 20:5	0,32	0,04	0,32	0,05	0,31	0,07	0,30	0,03	0,37	0,02	0,34	0,07	0,33
C 22:4	0,80	0,11	0,93	0,08	0,78	0,08	0,86	0,03	0,92	0,30	0,92	0,06	0,87
C 22:6	0,03	0,05	0,07	0,07	n.d.	n.d.	0,04	0,06	0,08	0,08	0,04	0,07	0,05
∑ SFA	38,91		38,38		38,82		39,37		35,96		37,54		38,16
∑ MUFA	23,42		24,38		27,42		21,79		25,44		23,33		24,30
∑ PUFA	32,83		33,20		30,02		34,83		34,62		35,13		33,44

	MST												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	0,15	0,01	0,05	0,06	0,06	0,07	0,06	0,06	0,11	0,06	0,16	0,01	0,10
C 15	0,20	0,03	0,17	0,01	0,15	0,01	0,17	0,01	0,18	0,03	0,18	0,02	0,18
C 16	19,31	1,66	17,98	1,64	20,89	2,62	19,37	1,17	19,42	2,80	19,99	0,64	19,49
C 16:1 tr.	0,28	0,02	0,22	0,05	0,24	0,05	0,16	0,01	0,27	0,03	0,23	0,06	0,23
C 16:1 c9	1,02	0,15	0,75	0,10	0,89	0,24	0,58	0,06	0,99	0,26	0,89	0,22	0,85
C 17	0,56	0,01	0,49	0,04	0,51	0,01	0,54	0,03	0,54	0,08	0,54	0,05	0,53
C 17:1 tr.	0,67	0,04	0,55	0,11	0,48	0,26	0,42	0,06	0,70	0,14	0,52	0,12	0,56
C 17:1	0,68	0,18	0,65	0,28	0,73	0,38	0,53	0,05	0,59	0,36	0,63	0,05	0,64
C 18	17,67	0,90	16,37	1,27	16,46	1,49	15,47	0,80	15,87	1,56	17,09	1,25	16,49
C 18:1 tr.	0,49	0,13	0,35	0,08	0,42	0,20	0,36	0,08	0,40	0,11	0,38	0,03	0,40
C 18:1 c9	20,40	1,40	17,92	2,23	20,80	4,90	16,35	1,35	20,56	3,92	17,24	2,64	18,88
C 18:1 c11	2,45	0,25	2,22	0,59	2,52	0,31	2,48	0,26	2,17	0,45	2,49	0,29	2,39
C 18:2	19,02	0,82	22,28	1,97	18,66	1,88	22,45	2,18	19,39	2,02	21,53	1,67	20,56
C 18:3 c6	0,17	0,01	0,13	0,11	0,08	0,10	0,13	0,13	0,19	0,03	0,14	0,08	0,14
C 18:3 c9	0,74	0,08	0,78	0,08	0,67	0,13	0,82	0,09	0,72	0,10	0,76	0,09	0,75
C 20:2	0,18	0,03	0,22	0,03	0,18	0,02	0,27	0,03	0,21	0,06	0,23	0,05	0,22
C 20:3 c8	1,80	0,26	2,45	0,25	1,84	0,40	2,51	0,35	2,00	0,35	1,93	0,17	2,09
C 20:4	8,21	0,21	10,29	0,47	8,70	0,95	10,74	0,37	8,64	0,63	8,98	0,64	9,26
C 20:5	0,38	0,07	0,47	0,07	0,40	0,09	0,63	0,11	0,49	0,16	0,36	0,05	0,46
C 22:4	0,96	0,09	1,07	0,12	0,95	0,10	1,11	0,07	1,12	0,29	1,03	0,14	1,04
C 22:6	0,11	0,06	n.d.	n.d.	0,03	0,07	0,11	0,11	0,15	0,02	0,07	0,07	0,09
∑ SFA	37,89		35,06		38,07		35,61		36,12		37,96		36,79
∑ MUFA	25,99		22,66		26,08		20,88		25,68		22,38		23,95
∑ PUFA	31,57		37,69		31,51		38,77		32,91		35,03		34,58

all values expressed as percentage of total fatty acids

Table A-11: Young Bulls I – Mean and Standard Deviation of Phospholipids in Liver

	Liver												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	0,13	0,13	0,28	0,06	0,03	0,04	0,25	0,04	0,02	0,03	n.d.	n.d.	0,14
C 14:1 c9	n.d.	n.d.	0,03	0,05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,03
C 15	0,13	0,04	0,17	0,03	0,15	0,03	0,16	0,02	0,14	0,03	0,10	0,06	0,14
C 16	7,91	0,90	8,22	0,38	9,73	0,56	8,20	0,64	8,37	0,72	7,74	0,77	8,36
C 16:1 tr.	0,03	0,03	0,08	0,05	0,07	0,07	0,07	0,04	0,03	0,05	n.d.	n.d.	0,06
C 16:1 c9	0,37	0,07	0,41	0,11	0,49	0,14	0,37	0,05	0,44	0,03	0,39	0,07	0,41
C 17	0,99	0,06	0,99	0,08	0,95	0,07	1,01	0,08	1,03	0,13	0,97	0,13	0,99
C 17:1 tr.	0,20	0,02	0,23	0,04	0,24	0,05	0,20	0,02	0,26	0,04	0,16	0,01	0,22
C 17:1	0,22	0,04	0,21	0,01	0,17	0,03	0,22	0,01	0,20	0,04	0,23	0,03	0,21
C 18	34,46	0,98	33,43	0,83	31,98	0,59	34,75	0,79	34,64	0,40	34,44	0,92	33,95
C 18:1 tr.	0,79	0,18	1,02	0,27	0,75	0,16	0,79	0,12	0,76	0,17	0,97	0,15	0,85
C 18:1 c9	11,01	1,60	11,46	1,30	12,75	1,79	10,49	1,41	11,57	0,31	9,84	1,23	11,19
C 18:1 c11	1,08	0,17	1,34	0,16	1,33	0,14	1,06	0,10	1,14	0,13	1,19	0,10	1,19
C 18:2	14,69	1,43	11,97	1,01	14,24	2,26	13,58	1,43	15,51	0,59	14,86	1,06	14,14
C 18:3 c6	0,20	0,12	0,32	0,05	0,29	0,10	0,22	0,13	0,24	0,06	0,13	0,08	0,23
C 18:3 c9	0,61	0,07	0,45	0,05	0,60	0,15	0,59	0,20	0,71	0,12	0,70	0,06	0,61
C 20:2	0,25	0,04	0,23	0,03	0,25	0,05	0,25	0,04	0,29	0,10	0,31	0,08	0,26
C 20:3 c8	4,78	0,68	6,18	1,03	4,39	1,14	5,52	1,75	4,29	0,73	4,72	0,38	4,98
C 20:4	12,39	1,22	12,70	0,93	12,13	0,51	12,36	0,75	11,53	1,16	12,73	1,05	12,31
C 20:3 c11	0,37	0,01	0,17	0,17	0,26	0,15	0,34	0,04	0,35	0,04	0,38	0,03	0,31
C 20:5	0,46	0,10	0,49	0,04	0,44	0,02	0,45	0,10	0,44	0,02	0,42	0,08	0,45
C 22:4	2,01	0,21	1,93	0,10	1,74	0,55	1,89	0,19	1,71	0,27	2,24	0,42	1,92
C 22:6	1,01	0,25	1,07	0,04	0,85	0,08	1,07	0,26	0,88	0,17	1,09	0,10	1,00
∑ SFA	43,62		43,09		42,84		44,37		44,20		43,25		43,56
∑ MUFA	13,70		14,78		15,80		13,20		14,40		12,78		14,11
∑ PUFA	36,77		35,51		35,19		36,27		35,95		37,58		36,21

all values expressed as percentage of total fatty acids

Table A-12: Young Bulls I – Mean and Standard Deviation in Serum Fractions I

	Triglycerides												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	1,78	0,66	1,25	0,15	1,70	0,26	1,24	0,62	1,16	0,15	0,65	0,51	1,30
C 14:1 c9	n.d.	n.d.	0,18	0,18	n.d.	n.d.	n.d.	n.d.	0,41	0,24	n.d.	n.d.	0,30
C 15	n.d.	n.d.	0,38	0,38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,10	0,17	0,24
C 16	21,54	0,71	21,86	1,10	22,89	0,63	20,38	2,70	21,59	1,24	19,00	0,70	21,21
C 16:1 tr.	0,90	0,26	2,48	0,54	1,74	0,44	0,80	0,49	1,49	0,73	1,34	0,27	1,46
C 16:1 c9	1,70	0,15	1,93	0,45	1,43	0,42	1,67	0,52	1,51	0,72	1,65	0,32	1,65
C 17	1,61	0,34	0,82	0,50	0,67	0,16	0,39	0,19	1,03	0,08	1,28	0,14	0,97
C 17:1 tr.	0,80	0,15	0,21	0,23	0,18	0,27	0,32	0,20	0,66	0,08	0,64	0,04	0,47
C 17:1	1,39	0,37	1,13	0,67	1,33	0,78	0,93	0,57	1,48	0,28	1,54	0,07	1,30
C 18	12,59	1,11	15,48	1,78	14,41	2,85	15,64	1,41	14,34	2,00	12,07	2,21	14,09
C 18:1 tr.	n.d.	n.d.	0,14	0,14	0,06	0,06	0,11	0,11	0,09	0,10	0,08	0,08	0,10
C 18:1 c9	18,74	1,22	21,29	1,29	21,80	1,20	19,06	2,50	22,57	1,40	14,89	1,87	19,73
C 18:1 c11	1,23	0,33	0,76	0,45	1,11	0,13	1,71	1,68	0,64	0,39	1,09	1,03	1,09
C 18:2	12,68	0,73	12,42	1,37	14,10	1,06	14,86	4,22	11,36	2,43	13,54	1,25	13,16
C 18:3 c6	n.d.	n.d.	0,47	0,48	1,05	0,79	0,35	0,30	0,54	0,04	0,79	0,09	0,64
C 18:3 c9	2,54	0,52	1,47	0,25	1,47	0,41	1,61	0,73	1,51	0,16	1,66	0,24	1,71
C 20:1 c11	n.d.	n.d.	n.d.	n.d.	0,51	0,18	1,20	0,44	0,61	0,18	0,72	0,11	0,76
C 20:3 c8	1,41	0,84	1,23	0,15	1,19	0,14	0,74	0,40	0,81	0,11	0,56	0,88	0,99
C 20:4	1,41	0,22	1,53	0,19	1,86	0,54	1,42	0,94	0,99	0,17	1,20	0,33	1,40
C 22:4	1,28	0,22	0,95	0,41	0,94	0,13	0,28	0,29	0,70	0,19	1,13	0,20	0,88
C 22:6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,03	0,05	n.d.	n.d.	n.d.	n.d.	0,03
∑ SFA	37,52		39,79		39,67		37,65		38,12		33,10		37,64
∑ MUFA	24,76		28,12		28,16		25,80		29,46		21,95		26,38
∑ PUFA	19,32		18,07		20,61		19,29		15,91		18,88		18,68

	Phospholipids												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	n.d.	n.d.	0,03	0,06	0,14	0,14	0,15	0,20	0,05	0,08	0,03	0,05	0,08
C 14:1 c9	n.d.	n.d.	n.d.	n.d.	0,04	0,07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,04
C 15	0,25	0,06	0,27	0,02	0,22	0,13	0,19	0,11	0,30	0,17	0,26	0,06	0,25
C 16	12,96	0,67	11,55	0,52	14,09	1,21	13,39	1,09	12,92	1,01	12,63	0,88	12,92
C 16:1 tr.	0,28	0,05	0,29	0,02	0,67	0,05	0,14	0,08	0,22	0,13	0,09	0,09	0,28
C 16:1 c9	0,32	0,02	0,27	0,01	0,30	0,18	0,41	0,26	0,26	0,15	0,26	0,06	0,30
C 17	1,18	0,05	1,09	0,09	0,97	0,11	1,23	0,10	1,16	0,11	1,16	0,15	1,13
C 17:1 tr.	0,28	0,04	0,27	0,03	0,20	0,20	0,19	0,12	0,24	0,14	0,17	0,11	0,23
C 17:1	n.d.	n.d.	0,05	0,09	n.d.	n.d.	n.d.	n.d.	0,02	0,03	0,05	0,09	0,04
C 18	30,22	0,55	29,60	0,61	28,63	0,77	30,31	1,12	30,05	1,12	30,09	1,17	29,82
C 18:1 tr.	0,68	0,06	0,76	0,13	0,26	0,26	0,50	0,29	0,63	0,15	0,78	0,12	0,60
C 18:1 c9	13,75	0,99	14,78	0,36	16,00	1,49	13,37	1,43	14,62	0,84	11,52	2,07	14,01
C 18:1 c11	0,96	0,12	0,92	0,10	1,04	0,19	1,12	0,20	0,89	0,07	0,91	0,13	0,97
C 18:2	23,53	1,46	24,32	2,17	24,35	0,94	24,59	1,77	24,53	0,98	27,64	2,45	24,83
C 18:3 c6	0,14	0,25	0,05	0,09	0,36	0,49	0,06	0,11	0,05	0,09	n.d.	n.d.	0,13
C 18:3 c9	0,62	0,15	0,59	0,14	0,42	0,28	0,64	0,25	0,72	0,12	0,70	0,04	0,62
C 20:1 c11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,07	0,12	0,01	0,02	n.d.	n.d.	0,04
C 20:3 c8	4,01	0,78	5,12	1,02	3,42	1,05	4,23	1,05	3,34	0,45	3,27	0,57	3,90
C 20:4	5,33	0,85	6,05	0,77	5,46	0,62	5,05	0,92	4,28	0,48	5,07	0,68	5,21
C 20:5	0,13	0,13	0,05	0,09	0,03	0,06	0,11	0,11	0,06	0,10	n.d.	n.d.	0,08
C 22:4	1,15	0,12	1,14	0,10	1,01	0,35	1,00	0,11	0,88	0,15	n.d.	n.d.	1,04
C 22:6	0,29	0,19	0,29	0,17	0,27	0,16	0,28	0,18	0,07	0,12	0,34	0,05	0,26
∑ SFA	44,61		42,54		44,05		45,27		44,48		44,17		44,19
∑ MUFA	16,27		17,34		18,51		15,80		16,89		13,78		16,43
∑ PUFA	35,20		37,61		35,32		35,96		33,93		37,02		35,84

all values expressed as percentage of total fatty acids

Table A-13: Young Bulls I – Mean and Standard Deviation in Serum Fractions II

	Cholesterol esters												
Fatty Acid	Group A 80		Group A 120		Group B 80		Group B 120		Group C 80		Group C 120		total
	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean	dev	mean
C 14	0,80	0,02	0,86	0,05	0,94	0,04	0,89	0,09	0,94	0,10	0,89	0,05	0,89
C 14:1 c9	0,36	0,05	0,39	0,03	0,40	0,05	0,36	0,05	0,43	0,05	0,34	0,03	0,38
C 15	0,56	0,05	0,61	0,08	0,62	0,07	0,65	0,11	0,66	0,06	0,60	0,08	0,62
C 16	7,81	0,73	9,76	0,59	9,54	0,92	9,55	0,27	8,05	0,92	8,21	0,31	8,82
C 16:1 tr.	1,00	0,31	0,78	0,08	0,88	0,03	0,92	0,29	0,83	0,08	0,70	0,02	0,85
C 16:1 c9	0,89	0,54	1,34	0,19	1,39	0,40	1,07	0,12	1,34	0,20	1,18	0,29	1,20
C 17	0,51	0,09	0,54	0,07	0,47	0,17	0,55	0,17	0,67	0,06	0,34	0,04	0,51
C 17:1 tr.	0,53	0,05	0,51	0,07	0,52	0,13	0,37	0,06	0,46	0,11	0,42	0,08	0,47
C 18	3,93	0,86	6,22	0,84	5,38	1,22	7,05	2,11	4,98	0,77	5,09	1,34	5,44
C 18:1 tr.	0,26	0,06	0,26	0,13	0,16	0,10	0,33	0,20	0,20	0,24	0,38	0,19	0,27
C 18:1 c9	6,76	1,01	8,98	0,78	8,01	1,19	7,14	0,69	6,11	0,42	6,14	0,83	7,19
C 18:1 c11	0,48	0,05	0,64	0,07	0,56	0,08	0,58	0,03	0,42	0,03	0,49	0,06	0,53
C 18:2	55,84	3,37	55,09	0,87	54,04	4,80	53,84	2,53	57,85	1,00	56,49	2,06	55,53
C 18:3 c6	1,07	0,36	1,51	0,48	1,03	0,39	0,99	0,49	0,94	0,33	0,60	0,14	1,02
C 18:3 c9	3,18	0,69	2,82	0,36	2,92	0,98	2,67	0,64	3,33	0,47	3,22	0,41	3,02
C 20:3 c8	0,47	0,12	0,62	0,21	0,57	0,13	0,51	0,16	0,44	0,01	0,45	0,09	0,51
C 20:4	2,26	0,38	2,86	0,50	2,68	0,60	2,40	0,41	2,00	0,15	2,36	0,35	2,43
C 20:5	0,09	0,09	0,17	0,04	0,13	0,08	0,08	0,08	n.d.	n.d.	0,04	0,07	0,10
C 22:6	0,24	0,14	0,20	0,12	0,17	0,10	0,25	0,15	0,17	0,17	0,51	0,38	0,26
∑ SFA	13,61		17,99		16,95		18,69		15,30		15,13		16,28
∑ MUFA	10,28		12,90		11,92		10,77		9,79		9,65		10,89
∑ PUFA	63,15		63,27		61,54		60,74		64,73		63,67		62,85

all values expressed as percentage of total fatty acids

Table A-14 : Young Bulls II - Mean and Standard Deviation of Total Fat in Tissues

MLD

Fatty Acid	Group D 180 mean	Group D 180 dev	Group D 270 mean	Group D 270 dev	Group C mean	Group C dev	total mean
C 14	2,48	0,32	2,58	0,39	2,58	0,37	2,55
C 14:1 tr.	0,10	0,06	0,10	0,08	0,12	0,02	0,11
C 14:1 c9	0,49	0,12	0,61	0,19	0,61	0,22	0,57
C 15	0,25	0,06	0,29	0,04	0,27	0,02	0,27
C 15:1	0,14	0,04	0,18	0,04	0,16	0,03	0,16
C 16	26,61	1,22	26,24	1,71	26,47	0,98	26,44
C 16:1 tr.	0,42	0,05	0,45	0,07	0,43	0,03	0,43
C 16:1 c9	3,43	0,56	3,75	0,61	3,46	0,67	3,55
C 17	0,75	0,10	0,77	0,10	0,80	0,07	0,78
C 17:1	0,73	0,10	0,79	0,08	0,79	0,05	0,77
C 18	16,08	1,33	14,83	1,60	15,88	1,49	15,60
C 18:1 tr.	0,18	0,11	0,16	0,06	0,13	0,06	0,16
C 18:1 c9	37,16	1,99	36,20	2,95	37,85	1,50	37,07
C 18:1 c11	1,50	0,19	1,60	0,12	1,57	0,21	1,56
C 18:2	4,98	1,39	5,81	2,08	4,67	1,09	5,16
C 18:3 c9	0,18	0,05	0,21	0,05	0,18	0,03	0,19
C 20:1 c11	0,47	0,08	0,43	0,11	0,38	0,12	0,43
C 20:3 c8	0,42	0,10	0,53	0,37	0,34	0,11	0,43
C 20:3/20:4	1,79	0,55	2,29	1,35	1,36	0,70	1,81
C 22:2/20:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 22:4	0,28	0,05	0,45	0,27	0,32	0,10	0,35
C 22:5	0,26	0,07	0,38	0,21	0,24	0,08	0,29
Σ SFA	46,18		44,72		46,01		45,64
Σ MUFA	44,62		44,27		45,50		44,80
Σ PUFA	7,93		9,67		7,12		8,24

MST

Fatty Acid	Group D 180 mean	Group D 180 dev	Group D 270 mean	Group D 270 dev	Group C mean	Group C dev	total mean
C 14	2,14	0,44	1,74	0,69	1,98	0,66	1,95
C 14:1 tr.	0,13	0,03	0,10	0,06	0,11	0,06	0,11
C 14:1 c9	0,48	0,13	0,48	0,37	0,54	0,23	0,50
C 15	0,30	0,05	0,28	0,07	0,29	0,05	0,29
C 15:1	0,17	0,02	0,17	0,04	0,17	0,03	0,17
C 16	24,53	1,50	22,86	2,59	23,92	2,08	23,77
C 16:1 tr.	0,52	0,02	0,50	0,05	0,49	0,05	0,51
C 16:1 c9	3,37	0,43	3,29	1,01	3,34	0,78	3,33
C 17	0,86	0,13	0,74	0,16	0,81	0,15	0,81
C 17:1	0,92	0,06	0,90	0,11	0,89	0,05	0,90
C 18	14,80	1,77	13,30	2,05	14,23	2,07	14,11
C 18:1 tr.	0,13	0,05	0,29	0,24	0,23	0,22	0,21
C 18:1 c9	38,41	1,53	36,87	4,51	38,18	1,60	37,82
C 18:1 c11	1,62	0,17	1,84	0,29	1,85	0,45	1,77
C 18:2	5,15	1,36	7,54	3,41	6,07	2,31	6,25
C 18:3 c9	0,20	0,04	0,28	0,13	0,23	0,06	0,24
C 20:1 c11	0,47	0,06	0,44	0,12	0,49	0,10	0,47
C 20:3 c8	0,51	0,20	0,87	0,59	0,56	0,28	0,65
C 20:3/20:4	2,19	0,90	3,74	2,15	2,45	1,28	2,79
C 22:2/20:5	0,07	0,07	0,17	0,15	0,07	0,11	0,10
C 22:4	0,47	0,21	0,77	0,45	0,53	0,25	0,59
C 22:5	0,35	0,13	0,66	0,37	0,42	0,23	0,48
Σ SFA	42,62		38,92		41,23		40,93
Σ MUFA	46,22		44,88		46,31		45,80
Σ PUFA	8,93		14,03		10,33		11,10

Liver

Fatty Acid	Group D 180 mean	Group D 180 dev	Group D 270 mean	Group D 270 dev	Group C mean	Group C dev	total mean
C 14	0,35	0,04	0,33	0,08	0,40	0,05	0,36
C 14:1 tr.	0,05	0,06	0,11	0,06	0,09	0,08	0,08
C 14:1 c9	0,26	0,04	0,31	0,05	0,28	0,04	0,29
C 15	0,22	0,02	0,24	0,03	0,26	0,02	0,24
C 15:1	0,11	0,06	0,12	0,07	0,13	0,07	0,12
C 16	8,09	0,73	8,17	0,82	9,14	0,87	8,46
C 16:1 tr.	0,42	0,04	0,38	0,06	0,46	0,06	0,42
C 16:1 c9	0,62	0,17	0,58	0,15	0,76	0,15	0,65
C 17	1,09	0,11	1,05	0,10	1,06	0,11	1,07
C 17:1	0,43	0,03	0,41	0,07	0,45	0,03	0,43
C 18	32,94	0,74	32,47	0,60	31,00	1,34	32,14
C 18:1 tr.	1,11	0,25	1,25	0,51	1,15	0,43	1,17
C 18:1 c9	11,40	0,99	11,02	1,09	12,06	0,82	11,49
C 18:1 c11	1,20	0,10	1,18	0,07	1,22	0,13	1,20
C 18:2	12,32	1,77	12,45	1,74	12,67	1,84	12,48
C 18:3 c6	0,15	0,18	0,17	0,15	0,24	0,14	0,19
C 20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 18:3 c9	0,42	0,11	0,42	0,09	0,47	0,12	0,43
C 20:1 c11	0,34	0,05	0,33	0,06	0,38	0,09	0,35
C 21	0,21	0,07	0,19	0,05	0,19	0,05	0,19
C 20:2	0,30	0,05	0,33	0,06	0,30	0,03	0,31
C 20:3 c8	5,10	1,46	5,38	1,05	5,19	0,85	5,23
C 20:3/20:4	12,13	0,69	12,21	0,49	11,75	0,77	12,03
C 22:2/20:5	0,39	0,10	0,40	0,08	0,40	0,07	0,40
C 22:4	3,29	0,57	3,50	0,52	3,02	0,13	3,27
C 22:5	2,55	0,21	2,65	0,40	2,57	0,26	2,59
C 22:6	1,11	0,15	1,06	0,16	1,01	0,15	1,06
Σ SFA	42,89		42,45		42,04		42,46
Σ MUFA	15,94		15,69		16,98		16,20
Σ PUFA	37,77		38,56		37,60		37,98

Kidney

Fatty Acid	Group D 180 mean	Group D 180 dev	Group D 270 mean	Group D 270 dev	Group C mean	Group C dev	total mean
C 14	3,18	0,20	3,32	0,30	2,97	0,30	3,16
C 14:1 tr.	0,21	0,03	0,22	0,02	0,19	0,03	0,21
C 14:1 c9	0,40	0,07	0,40	0,04	0,33	0,05	0,38
C 15	0,43	0,06	0,44	0,07	0,34	0,03	0,40
C 15:1	0,33	0,07	0,36	0,05	0,29	0,05	0,33
C 16	25,31	0,94	26,02	1,11	24,12	1,49	25,15
C 16:1 tr.	0,61	0,07	0,59	0,04	0,54	0,05	0,58
C 16:1 c9	1,69	0,34	1,57	0,19	1,49	0,27	1,59
C 17	1,21	0,08	1,24	0,06	1,19	0,07	1,21
C 17:1	0,62	0,08	0,60	0,04	0,59	0,06	0,60
C 18	28,14	3,65	29,22	1,77	30,10	2,54	29,15
C 18:1 tr.	2,82	0,69	2,84	0,74	3,09	0,65	2,92
C 18:1 c9	29,14	3,27	27,63	1,12	29,22	1,88	28,66
C 18:1 c11	1,11	0,07	1,06	0,11	1,08	0,12	1,08
C 18:2	1,70	0,33	1,66	0,25	1,63	0,12	1,66
C 18:3 c6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 20	0,21	0,03	0,22	0,01	0,20	0,02	0,21
C 18:3 c9	0,13	0,03	0,11	0,06	0,12	0,01	0,12
C 20:1 c11	0,36	0,10	0,33	0,11	0,32	0,06	0,34
C 21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 20:2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 20:3 c8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 20:3/20:4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 22:2/20:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 22:4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 22:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 22:6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ SFA	58,47		60,46		58,92		59,29
Σ MUFA	37,30		35,61		37,13		36,68
Σ PUFA	1,82		1,77		1,76		1,78

all values expressed as percentage of total fatty acids

Table A-15 : Young Bulls II - Mean and Standard Deviation of Phospholipids in Tissues

	MLD						
Fatty Acid	Group D 180		Group D 270		Group C		total
	mean	dev	mean	dev	mean	dev	mean
C 14	0,11	0,01	0,11	0,02	0,12	0,01	0,11
C 15	0,16	0,01	0,16	0,02	0,16	0,02	0,16
C 15:1	0,09	0,01	0,11	0,02	0,10	0,01	0,10
C 16	18,17	1,19	18,39	1,48	18,50	1,05	18,35
C 16:1 tr.	0,60	0,05	0,56	0,04	0,57	0,04	0,58
C 16:1 c9	1,69	0,45	1,54	0,19	1,61	0,11	1,61
C 17	0,44	0,04	0,45	0,04	0,45	0,03	0,45
C 17:1	0,83	0,13	0,80	0,08	0,86	0,05	0,83
C 18	13,68	0,41	13,97	0,74	13,65	0,80	13,77
C 18:1 c9	25,83	3,42	24,54	2,33	25,60	1,81	25,32
C 18:1 c11	2,21	0,23	2,25	0,15	2,33	0,30	2,26
C 18:2	19,04	2,58	19,32	1,59	19,39	1,57	19,25
C 18:3 c6	0,17	0,01	0,17	0,05	0,17	0,03	0,17
C 18:3 c9	0,51	0,12	0,54	0,06	0,53	0,05	0,53
C 20:1 c11	0,35	0,03	0,31	0,09	0,32	0,10	0,33
C 20:2	0,18	0,04	0,21	0,02	0,18	0,03	0,19
C 20:3 c8	1,86	0,20	1,79	0,44	1,74	0,25	1,79
C 20:3/20:4	9,01	0,98	9,17	1,28	8,33	0,63	8,84
C 22:2/20:5	0,35	0,10	0,43	0,16	0,35	0,04	0,37
C 22:4	1,19	0,14	1,48	0,26	1,56	0,09	1,41
C 22:5	1,10	0,15	1,26	0,32	1,11	0,07	1,15
C 22:6	0,14	0,04	0,15	0,04	0,13	0,03	0,14
∑ SFA	32,55		33,08		32,88		32,84
∑ MUFA	31,60		30,12		31,39		31,04
∑ PUFA	33,53		34,52		33,50		33,85

	MST						
	Group D 180		Group D 270		Group C		total
	mean	dev	mean	dev	mean	dev	mean
	0,11	0,01	0,10	0,02	0,12	0,01	0,11
	0,18	0,01	0,18	0,03	0,21	0,03	0,19
	0,11	0,01	0,13	0,02	0,13	0,02	0,12
	17,43	0,94	17,36	2,22	19,91	1,81	18,23
	0,61	0,06	0,60	0,07	0,62	0,05	0,61
	1,60	0,59	1,39	0,23	1,49	0,17	1,49
	0,49	0,09	0,48	0,03	0,51	0,02	0,49
	0,85	0,17	0,80	0,09	0,83	0,04	0,82
	13,95	0,78	13,67	1,00	15,31	0,80	14,31
	24,82	3,59	24,76	3,15	24,65	1,26	24,75
	1,86	0,18	1,82	0,23	2,00	0,19	1,89
	17,92	2,71	18,02	2,42	16,96	1,24	17,63
	0,16	0,01	0,18	0,03	0,16	0,03	0,17
	0,50	0,13	0,53	0,08	0,48	0,03	0,50
	0,35	0,04	0,31	0,05	0,35	0,07	0,34
	0,20	0,06	0,22	0,04	0,19	0,02	0,21
	2,12	0,42	2,06	0,47	1,71	0,26	1,97
	9,95	0,88	10,27	1,18	8,42	0,74	9,54
	0,47	0,18	0,57	0,20	0,41	0,05	0,49
	1,86	0,23	1,86	0,28	1,53	0,14	1,75
	1,41	0,33	1,51	0,38	1,17	0,09	1,36
	0,17	0,06	0,21	0,06	0,15	0,03	0,18
	32,16		31,78		36,05		33,33
	30,20		29,81		30,06		30,02
	34,77		35,43		31,18		33,79

	Liver						
Fatty Acid	Group D 180		Group D 270		Group C		total
	mean	dev	mean	dev	mean	dev	mean
C 15	0,14	0,01	0,15	0,03	0,15	0,03	0,15
C 15:1	0,10	0,02	0,11	0,03	0,11	0,03	0,10
C 16	6,51	0,44	6,63	0,58	7,25	0,48	6,80
C 16:1 tr.	0,27	0,10	0,24	0,09	0,32	0,06	0,28
C 16:1 c9	0,39	0,10	0,39	0,10	0,45	0,05	0,41
C 17	1,08	0,10	1,06	0,12	1,07	0,11	1,07
C 17:1	0,42	0,04	0,42	0,07	0,43	0,04	0,43
C 18	35,13	0,82	34,48	0,50	33,58	1,13	34,40
C 18:1 tr.	0,18	0,22	0,07	0,06	0,07	0,06	0,11
C 18:1 c9	11,29	1,08	11,20	1,23	11,90	0,66	11,46
C 18:1 c11	1,11	0,09	1,08	0,09	1,17	0,14	1,12
C 18:2	12,76	2,07	12,78	1,87	13,04	2,01	12,86
C 18:3 c6	0,24	0,12	0,23	0,11	0,28	0,11	0,25
C 18:3 c9	0,43	0,12	0,44	0,09	0,46	0,13	0,44
C 20:1 c11	0,10	0,05	0,09	0,03	0,11	0,02	0,10
C 20:2	0,30	0,05	0,33	0,06	0,31	0,03	0,31
C 20:3 c8	5,44	1,55	5,73	1,13	5,62	0,89	5,60
C 20:3/20:4	12,77	0,75	13,00	0,55	12,64	0,66	12,81
C 22:2/20:5	0,42	0,09	0,44	0,08	0,45	0,09	0,44
C 22:4	3,22	0,53	3,48	0,54	3,04	0,09	3,25
C 22:5	2,52	0,22	2,63	0,40	2,59	0,27	2,58
C 22:6	1,15	0,15	1,12	0,14	1,08	0,18	1,12
∑ SFA	42,87		42,32		42,06		42,42
∑ MUFA	13,86		13,60		14,57		14,01
∑ PUFA	39,27		40,18		39,51		39,65

all values expressed as percentage of total fatty acids

Table A-16 : Young Bulls II - Mean and Standard Deviation in Serum Fractions

	Triglycerides						
Fatty Acid	Group D 180		Group D 270		Group C		total
	mean	dev	mean	dev	mean	dev	mean
C 12	0,14	0,12	0,33	0,11	0,14	0,12	0,20
C 14	1,74	0,17	1,97	0,39	1,92	0,28	1,88
C 14:1 tr.	1,08	0,45	0,80	0,20	0,72	0,08	0,87
C 14:1 c9	1,13	0,17	1,25	0,19	1,21	0,27	1,20
C 15	1,29	0,21	1,48	0,10	1,49	0,13	1,42
C 15:1	0,63	0,21	0,74	0,14	0,70	0,09	0,69
C 16	22,61	1,44	22,52	0,75	24,07	1,60	23,07
C 16:1 tr.	1,93	0,37	1,85	0,24	2,25	0,17	2,01
C 16:1 c9	1,71	0,69	1,11	0,27	1,48	0,31	1,43
C 17	1,97	0,25	2,00	0,15	1,86	0,14	1,94
C 17:1	0,91	0,43	0,54	0,20	0,53	0,19	0,66
C 18	29,20	2,64	34,04	3,98	28,36	3,47	30,53
C 18:1 tr.	1,65	0,55	2,56	0,93	1,83	0,64	2,01
C 18:1 c9	16,06	1,61	12,87	1,61	16,59	2,55	15,17
C 18:1 c11	1,41	0,24	1,63	0,20	1,60	0,19	1,55
C 18:2	5,22	0,43	4,81	1,05	6,00	0,72	5,34
C 18:3 c6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 20	0,33	0,16	0,50	0,06	0,41	0,07	0,41
C 18:3 c9	0,46	0,07	0,46	0,14	0,67	0,10	0,53
C 20:1 c11	0,41	0,10	0,37	0,11	0,49	0,11	0,42
C 20:2	0,40	0,50	n.d.	n.d.	n.d.	n.d.	0,40
C 20:3 c8	0,34	0,30	0,36	0,08	0,47	0,16	0,39
C 20:3/20:4	0,59	0,10	0,54	0,10	0,66	0,14	0,60
C 22:2/20:5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 24	n.d.	n.d.	n.d.	n.d.	0,33	0,04	0,33
C 22:4	0,14	0,17	n.d.	n.d.	n.d.	n.d.	0,14
C 22:5	0,15	0,16	0,23	0,04	0,25	0,05	0,21
C 22:6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑ SFA	57,28		63,20		58,62		59,70
∑ MUFA	26,91		23,72		27,40		26,01
∑ PUFA	7,30		6,40		8,37		7,36

	Phospholipids						
	Group D 180		Group D 270		Group C		total
	mean	dev	mean	dev	mean	dev	mean
C 12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 14	0,14	0,02	0,20	0,04	0,15	0,05	0,16
C 14:1 tr.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 14:1 c9	0,18	0,04	0,23	0,09	0,24	0,06	0,22
C 15	0,26	0,02	0,30	0,03	0,29	0,05	0,28
C 15:1	0,20	0,05	0,23	0,04	0,23	0,07	0,22
C 16	11,22	0,74	12,11	0,45	12,07	0,61	11,80
C 16:1 tr.	0,49	0,05	0,38	0,16	0,44	0,09	0,44
C 16:1 c9	0,33	0,02	0,36	0,08	0,35	0,04	0,34
C 17	1,51	0,16	1,43	0,13	1,42	0,10	1,45
C 17:1	0,40	0,04	0,39	0,05	0,38	0,04	0,39
C 18	30,27	1,56	29,02	0,90	29,07	1,22	29,45
C 18:1 tr.	1,15	0,23	1,14	0,39	1,23	0,24	1,17
C 18:1 c9	13,00	1,34	12,99	1,64	12,97	0,75	12,99
C 18:1 c11	1,06	0,13	0,93	0,09	1,12	0,20	1,04
C 18:2	25,19	2,95	26,13	2,00	25,07	2,79	25,46
C 18:3 c6	0,17	0,08	0,15	0,10	0,20	0,08	0,17
C 20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 18:3 c9	0,58	0,09	0,61	0,04	0,57	0,11	0,59
C 20:1 c11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 20:2	0,20	0,04	0,20	0,02	0,20	0,02	0,20
C 20:3 c8	3,37	1,13	3,35	0,94	3,72	0,89	3,48
C 20:3/20:4	4,35	0,53	4,20	0,49	4,35	0,63	4,30
C 22:2/20:5	0,15	0,04	0,13	0,08	0,18	0,05	0,15
C 24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 22:4	1,28	0,20	1,31	0,25	1,26	0,13	1,28
C 22:5	0,88	0,15	0,89	0,14	1,03	0,20	0,93
C 22:6	0,29	0,08	0,27	0,05	0,28	0,09	0,28
∑ SFA	43,40		43,07		42,99		43,15
∑ MUFA	16,81		16,65		16,95		16,81
∑ PUFA	36,45		37,24		36,86		36,85

	Cholesterol esters						
Fatty Acid	Group D 180		Group D 270		Group C		total
	mean	dev	mean	dev	mean	dev	mean
C 10	0,13	0,04	0,11	0,06	0,16	0,03	0,13
C 12	0,08	0,08	0,10	0,09	0,07	0,08	0,08
C 14	1,02	0,16	0,97	0,29	0,96	0,08	0,98
C 14:1 tr.	0,33	0,06	0,33	0,12	0,33	0,03	0,33
C 14:1 c9	1,10	0,17	1,02	0,25	1,01	0,11	1,04
C 15	0,59	0,07	0,53	0,10	0,57	0,05	0,56
C 15:1	0,40	0,06	0,41	0,08	0,41	0,06	0,41
C 16	5,82	0,41	5,75	1,23	5,63	1,30	5,73
C 16:1 tr.	1,07	0,09	0,94	0,16	1,03	0,11	1,01
C 16:1 c9	1,55	0,14	1,48	0,33	1,40	0,14	1,48
C 17	1,30	0,63	1,91	1,29	2,70	0,79	1,97
C 17:1	0,50	0,05	0,49	0,07	0,46	0,04	0,48
C 18	3,53	3,89	2,42	1,45	2,24	2,43	2,73
C 18:1 tr.	0,02	0,06	0,13	0,20	0,07	0,17	0,07
C 18:1 c9	6,02	0,75	6,70	1,00	6,11	1,64	6,28
C 18:1 c11	0,49	0,11	0,49	0,08	0,47	0,12	0,49
C 18:2	65,22	4,23	65,51	4,19	65,12	5,91	65,28
C 18:3 c6	1,31	0,64	1,34	0,81	1,58	0,73	1,41
C 18:3 c9	3,10	0,22	2,86	0,35	3,22	1,06	3,06
C 20:3 c8	0,65	0,21	0,68	0,14	0,63	0,11	0,65
C 20:3/20:4	2,88	0,56	2,87	0,39	2,66	0,46	2,80
∑ SFA	12,64		11,97		12,47		12,36
∑ MUFA	11,67		12,14		11,30		11,70
∑ PUFA	73,17		73,25		73,20		73,21

all values expressed as percentage of total fatty acids

Table A-17 : Steers - Mean and Standard Deviation of Total Fat in Tissues

Fatty Acid	MLD					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 12	0,06	0,01	0,18	0,11	0,41	0,26
C 14	2,72	0,50	2,40	0,36	2,85	0,42
C 14:1 c9	0,73	0,29	0,44	0,14	0,48	0,13
C 15	0,23	0,05	0,60	0,09	0,33	0,06
C 15:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 16	28,36	1,61	24,22	1,15	25,57	1,31
C 16:1 tr.	0,19	0,14	0,45	0,19	0,24	0,20
C 16:1 c9	3,65	0,67	2,39	0,63	3,32	0,63
C 17	0,69	0,07	1,26	0,25	0,74	0,21
C 17:1	0,56	0,06	0,78	0,16	0,64	0,13
C 18	13,97	1,25	19,31	4,13	12,84	2,70
C 18:1 tr.	1,08	0,25	2,36	0,35	1,35	0,49
C 18:1 c9	40,45	2,51	33,23	3,10	36,85	1,93
C 18:1 c11	2,04	0,43	1,75	0,47	2,27	0,47
C 18:2	2,33	0,43	4,42	1,33	6,55	2,36
C 20	0,25	0,14	0,43	0,21	0,34	0,17
C 18:3 c9	0,15	0,03	1,51	0,35	0,76	0,11
C 20:1 c11	0,10	0,08	0,06	0,06	0,16	0,12
C 20:2	n.d.	n.d.	0,04	0,06	0,15	0,11
C 20:3 c8	0,31	0,05	0,31	0,08	0,47	0,11
C 20:4	0,88	0,19	0,96	0,35	1,41	0,33
C 20:5	0,03	0,03	0,32	0,15	0,33	0,11
C 22:4	0,24	0,05	0,09	0,03	0,19	0,04
C 22:5	0,19	0,05	0,75	0,24	0,87	0,16
C 22:6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑ SFA	46,29		48,39		43,08	
∑ MUFA	48,79		41,46		45,31	
∑ PUFA	4,13		8,39		10,75	

Fatty Acid	Liver					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 14	0,54	0,12	0,58	0,15	0,41	0,12
C 14:1 tr.	0,12	0,09	0,10	0,04	0,10	0,08
C 14:1 c9	0,09	0,05	0,06	0,05	n.d.	n.d.
C 15	0,25	0,02	0,43	0,06	0,26	0,03
C 15:1	0,11	0,02	0,09	0,01	0,09	0,05
C 16	11,68	1,70	14,20	2,02	10,53	1,06
C 16:1 tr.	0,48	0,11	0,63	0,13	0,37	0,02
C 16:1 c9	0,94	0,24	0,79	0,23	0,53	0,08
C 17	1,04	0,11	1,61	0,15	1,09	0,13
C 17:1	0,36	0,09	0,40	0,08	0,38	0,02
C 18	28,69	2,12	25,57	1,78	29,39	1,02
C 18:1 tr.	0,93	0,13	1,42	0,19	1,02	0,15
C 18:1 c9	13,86	1,87	11,03	2,02	9,83	0,89
C 18:1 c11	1,33	0,11	0,88	0,07	1,13	0,07
C 18:2	7,38	0,78	6,74	0,27	7,57	0,98
C 18:3 c6	0,56	0,32	0,27	0,11	0,23	0,04
C 18:3 c9	0,35	0,04	2,92	0,44	0,70	0,10
C 20:1 c11	0,26	0,06	0,47	0,12	0,27	0,04
C 20:2	0,16	0,02	0,14	0,02	0,22	0,04
C 20:3 c8	7,28	1,48	3,76	0,68	6,89	0,80
C 20:4	11,14	0,67	6,70	1,08	9,58	0,71
C 20:5	0,56	0,04	4,03	0,61	1,99	0,42
C 22:4	3,83	0,44	0,94	0,22	1,85	0,39
C 22:5	3,45	0,27	10,88	1,25	8,93	0,58
C 22:6	1,28	0,23	2,61	0,50	4,27	0,33
∑ SFA	42,20		42,39		41,67	
∑ MUFA	18,47		15,88		13,71	
∑ PUFA	35,99		39,00		42,23	

Fatty Acid	Kidney					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 14	3,73	0,60	3,10	0,26	3,27	0,42
C 14:1 tr.	0,23	0,08	0,48	0,05	0,32	0,03
C 14:1 c9	0,36	0,13	0,16	0,04	0,30	0,13
C 15	0,40	0,12	0,93	0,06	0,57	0,05
C 15:1	0,26	0,07	0,35	0,04	0,27	0,04
C 16	28,07	3,01	24,25	1,37	24,78	1,05
C 16:1 tr.	0,53	0,07	0,84	0,07	0,61	0,02
C 16:1 c9	1,74	0,34	1,23	0,13	1,72	0,42
C 17	1,12	0,10	1,97	0,11	1,44	0,11
C 17:1	0,55	0,06	0,66	0,07	0,69	0,08
C 18	26,23	2,79	32,63	1,47	29,55	2,81
C 18:1 tr.	2,48	0,39	3,40	0,31	2,86	0,14
C 18:1 c9	29,33	4,25	23,00	0,90	27,88	2,59
C 18:1 c11	0,97	0,06	1,01	0,05	1,03	0,12
C 18:2	1,00	0,26	0,91	0,13	0,89	0,19
C 20	0,18	0,03	0,31	0,02	0,24	0,05
C 18:3 c9	0,13	0,02	0,80	0,11	0,38	0,06
C 20:1 c11	0,10	0,07	0,03	0,04	0,10	0,03
∑ SFA	59,73		63,19		59,85	
∑ MUFA	36,54		31,16		35,79	
∑ PUFA	1,13		1,72		1,27	

all values expressed as percentage of total fatty acids

Table A-18 : Steers - Mean and Standard Deviation of Phospholipids in Tissues

Fatty Acid	MLD					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 14	0,25	0,18	0,13	0,02	0,11	0,01
C 15	0,11	0,09	0,23	0,02	0,15	0,01
C 16	20,30	2,36	18,49	2,08	16,49	0,57
C 16:1 tr.	0,47	0,19	0,44	0,21	0,43	0,12
C 16:1 c9	1,60	0,25	1,07	0,15	1,43	0,19
C 17	0,51	0,04	0,70	0,04	0,52	0,02
C 17:1	0,73	0,13	0,91	0,18	0,94	0,17
C 18	11,72	0,42	13,03	0,86	11,49	0,77
C 18:1 tr.	0,12	0,19	0,37	0,07	0,25	0,12
C 18:1 c9	29,39	2,36	20,89	2,68	25,80	2,83
C 18:1 c11	3,04	0,78	2,92	0,55	2,83	0,34
C 18:2	14,52	1,36	15,75	2,10	15,71	1,46
C 18:3 c6	0,29	0,05	0,18	0,09	0,27	0,02
C 20	0,16	0,06	0,20	0,07	0,20	0,11
C 18:3 c9	0,65	0,11	5,83	0,94	2,33	0,29
C 20:1 c11	0,12	0,03	0,03	0,04	0,10	0,01
C 20:2	0,46	0,39	0,11	0,07	0,10	0,06
C 20:3 c8	2,26	0,39	1,86	0,23	2,36	0,20
C 20:4	8,33	1,18	7,22	0,52	8,44	0,64
C 20:5	0,32	0,14	2,22	0,59	1,97	0,47
C 22:4	1,97	0,13	0,63	0,05	0,95	0,12
C 22:5	1,30	0,41	4,39	0,50	4,93	0,26
C 22:6	0,23	0,06	0,70	0,22	0,84	0,09
∑ SFA	33,05		32,78		28,97	
∑ MUFA	35,46		26,64		31,77	
∑ PUFA	30,32		38,88		37,91	

Fatty Acid	Liver					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 15	0,15	0,04	0,20	0,02	0,16	0,02
C 16	8,99	0,63	9,64	0,81	8,78	0,87
C 16:1 tr.	0,32	0,04	0,28	0,03	0,27	0,03
C 16:1 c9	0,53	0,06	0,30	0,05	0,32	0,03
C 17	1,02	0,10	1,33	0,14	1,04	0,11
C 17:1	0,37	0,05	0,30	0,04	0,31	0,04
C 18	31,17	1,14	30,63	1,79	31,35	0,86
C 18:1 tr.	1,00	0,28	1,37	0,20	0,97	0,15
C 18:1 c9	11,53	1,21	8,36	1,36	8,23	0,58
C 18:1 c11	1,21	0,08	0,79	0,08	1,06	0,07
C 18:2	7,26	0,70	6,54	0,38	7,09	1,12
C 18:3 c6	0,42	0,09	0,25	0,10	0,24	0,05
C 20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C 18:3 c9	0,32	0,04	2,36	0,30	0,62	0,08
C 20:1 c11	0,26	0,07	0,38	0,09	0,25	0,03
C 20:2	0,16	0,02	0,16	0,02	0,22	0,04
C 20:3 c8	7,79	1,34	4,34	0,68	7,22	0,71
C 20:4	12,27	1,24	7,94	0,75	10,11	0,77
C 20:5	0,63	0,06	4,59	0,56	2,08	0,45
C 22:4	3,96	0,50	1,07	0,20	1,86	0,38
C 22:5	3,59	0,45	12,11	0,86	9,05	0,76
C 22:6	1,40	0,31	3,05	0,46	4,51	0,24
∑ SFA	41,34		41,79		41,33	
∑ MUFA	15,20		11,78		11,41	
∑ PUFA	37,77		42,40		42,99	

all values expressed as percentage of total fatty acids

Table A-19 : Steers - Mean and Standard Deviation in Serum Fractions

Fatty Acid	Triglycerides					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 10	0,06	0,09	0,14	0,16	n.d.	n.d.
C 12	0,27	0,04	0,31	0,24	0,09	0,10
C 14	2,15	0,25	1,93	0,44	1,82	0,24
C 14:1 tr.	0,58	0,10	0,61	0,09	0,64	0,18
C 14:1 c9	0,81	0,20	0,66	0,10	0,85	0,26
C 15	1,37	0,20	1,72	0,11	1,49	0,21
C 15:1	0,51	0,09	0,29	0,05	0,48	0,11
C 16	26,72	1,97	27,29	1,82	26,47	2,96
C 16:1 tr.	1,85	0,26	1,99	0,28	1,88	0,19
C 16:1 c9	1,66	0,22	1,03	0,15	1,38	0,44
C 17	1,70	0,12	3,92	0,83	2,42	0,39
C 17:1	0,45	0,06	0,38	0,11	0,46	0,11
C 18	28,39	4,10	26,39	4,80	29,00	5,54
C 18:1 tr.	3,34	0,78	2,34	0,41	2,53	0,99
C 18:1 c9	17,80	1,99	13,58	1,46	15,67	2,87
C 18:1 c11	1,39	0,09	0,97	0,09	1,35	0,14
C 18:2	3,22	0,47	3,52	0,68	3,84	0,91
C 18:3 c6	0,14	0,17	n.d.	n.d.	0,09	0,13
C 20	0,41	0,05	0,47	0,08	0,43	0,10
C 18:3 c9	0,37	0,05	2,61	0,42	0,92	0,20
C 20:3 c8	0,65	0,12	0,45	0,11	0,80	0,26
C 20:4	0,75	0,20	0,79	0,20	1,03	0,28
C 20:5	n.d.	n.d.	1,05	0,35	n.d.	n.d.
C 24	0,36	0,18	0,48	0,07	0,09	0,13
C 22:4	0,32	0,17	0,05	0,08	n.d.	n.d.
C 22:5	0,47	0,13	1,92	0,40	1,71	0,38
C 22:6	0,13	0,06	0,54	0,12	0,63	0,10
∑ SFA	61,43		62,65		61,80	
∑ MUFA	28,39		21,87		25,23	
∑ PUFA	6,04		10,93		9,01	

Fatty Acid	Phospholipids					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 14	0,18	0,04	0,14	0,04	0,15	0,03
C 15	0,29	0,04	0,37	0,04	0,31	0,04
C 15:1	0,16	0,02	0,11	0,03	0,17	0,04
C 16	13,80	0,50	14,89	1,43	13,82	1,02
C 16:1 tr.	0,45	0,04	0,42	0,02	0,44	0,04
C 16:1 c9	0,40	0,04	0,26	0,03	0,33	0,04
C 17	1,50	0,16	5,73	2,09	1,59	0,16
C 17:1	0,43	0,09	0,09	0,02	0,39	0,04
C 18	27,17	1,31	22,35	0,89	26,08	0,89
C 18:1 tr.	0,81	0,25	0,85	0,17	0,71	0,21
C 18:1 c9	14,96	1,58	10,66	1,24	13,46	0,68
C 18:1 c11	0,99	0,11	0,64	0,04	0,88	0,03
C 18:2	13,86	1,68	15,67	1,12	15,54	2,96
C 18:3 c6	0,32	0,05	0,27	0,09	0,20	0,12
C 18:3 c9	0,42	0,11	3,53	0,47	0,60	0,45
C 20:3 c8	7,48	0,96	3,60	0,56	6,58	0,73
C 20:4	6,67	0,43	5,09	0,54	5,56	1,08
C 20:5	0,48	0,06	3,81	0,36	1,23	0,19
C 22:4	2,48	0,43	0,59	0,12	1,08	0,23
C 22:5	2,02	0,35	6,32	0,83	4,65	0,97
C 22:6	0,51	0,10	1,02	0,24	1,55	0,25
∑ SFA	42,94		43,48		41,95	
∑ MUFA	18,20		13,04		16,38	
∑ PUFA	34,24		39,89		36,99	

Fatty Acid	Cholesterol ester					
	silage		pasture		mixed	
	mean	dev	mean	dev	mean	dev
C 10	0,08	0,04	0,05	0,03	0,13	0,13
C 14	0,81	0,13	0,67	0,10	1,04	0,11
C 14:1 tr.	0,57	0,06	0,67	0,11	0,56	0,08
C 14:1 c9	0,85	0,13	0,86	0,14	0,96	0,14
C 15	0,64	0,05	1,02	0,12	0,75	0,10
C 15:1	0,40	0,05	0,33	0,04	0,44	0,08
C 16	6,33	0,53	5,55	0,58	7,78	1,19
C 16:1 tr.	1,15	0,22	0,97	0,13	1,22	0,17
C 16:1 c9	1,86	0,34	1,22	0,14	1,74	0,19
C 17	0,43	0,15	0,25	0,05	0,46	0,09
C 17:1	0,53	0,15	0,41	0,05	0,54	0,05
C 18	2,36	1,05	1,20	0,19	3,91	4,12
C 18:1 c9	7,45	1,09	4,33	0,85	9,26	2,75
C 18:1 c11	0,57	0,08	0,35	0,06	0,59	0,09
C 18:2	51,91	4,93	43,73	3,53	51,99	4,18
C 18:3 c6	3,40	0,75	1,33	0,28	3,52	0,90
C 20	0,24	0,09	0,23	0,05	0,10	0,08
C 18:3 c9	3,04	0,30	26,28	1,83	4,32	0,49
C 18:4	0,31	0,09	0,86	0,19	0,38	0,08
C 20:3 c8	1,58	0,22	0,57	0,09	1,27	0,30
C 20:4	4,92	0,59	2,36	0,41	3,59	1,23
C 20:5	0,73	0,11	3,81	0,45	1,70	0,55
C 24	0,35	0,28	n.d.	n.d.	n.d.	n.d.
C 22:5	1,24	0,53	0,41	0,31	0,69	0,67
C 22:6	0,09	0,03	0,12	0,03	0,25	0,07
∑ SFA	11,24		8,97		14,16	
∑ MUFA	13,38		9,14		15,31	
∑ PUFA	67,23		79,47		67,71	

all values expressed as percentage of total fatty acids

8.7.3 Results of Multivariate Data Analysis

Figure A-1 : Young Bulls I – Scores-Plot of PLSR2 Data Analysis

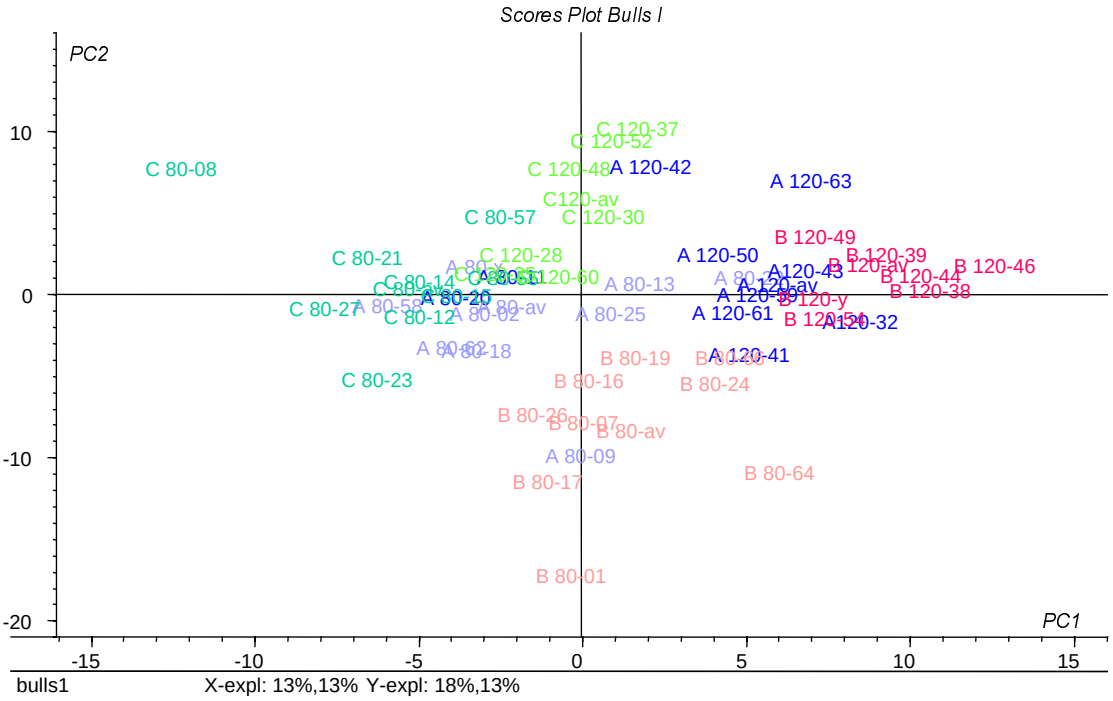


Figure A-2 : Young Bulls II – Scores-Plot of PLSR2 Data Analysis

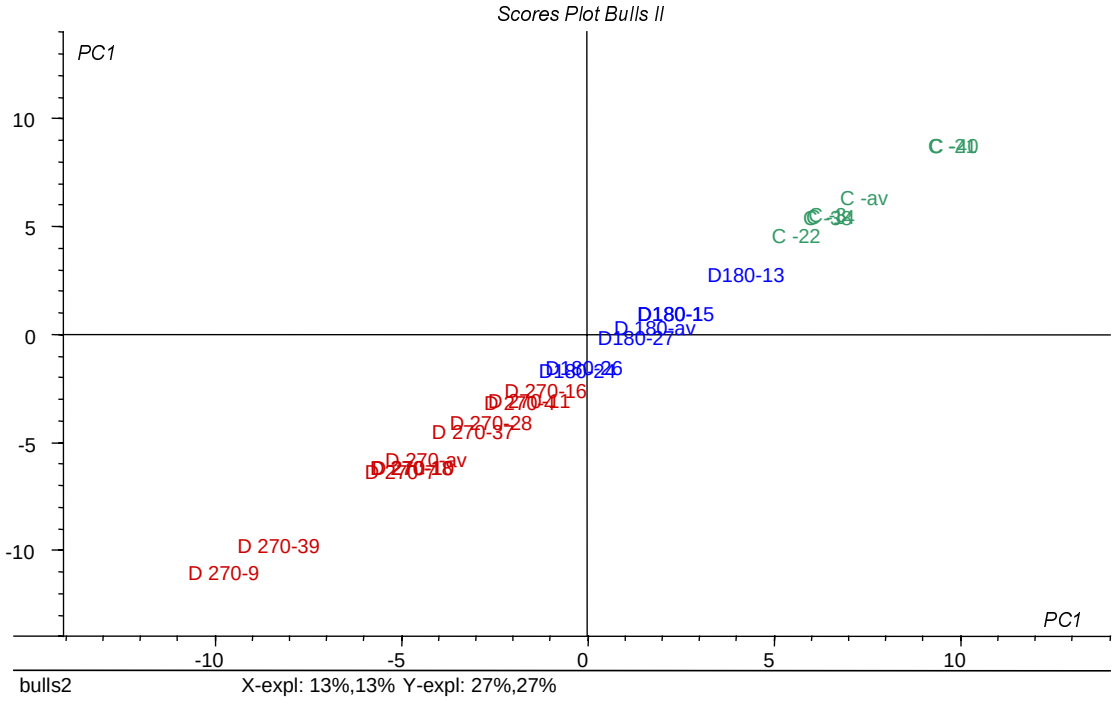
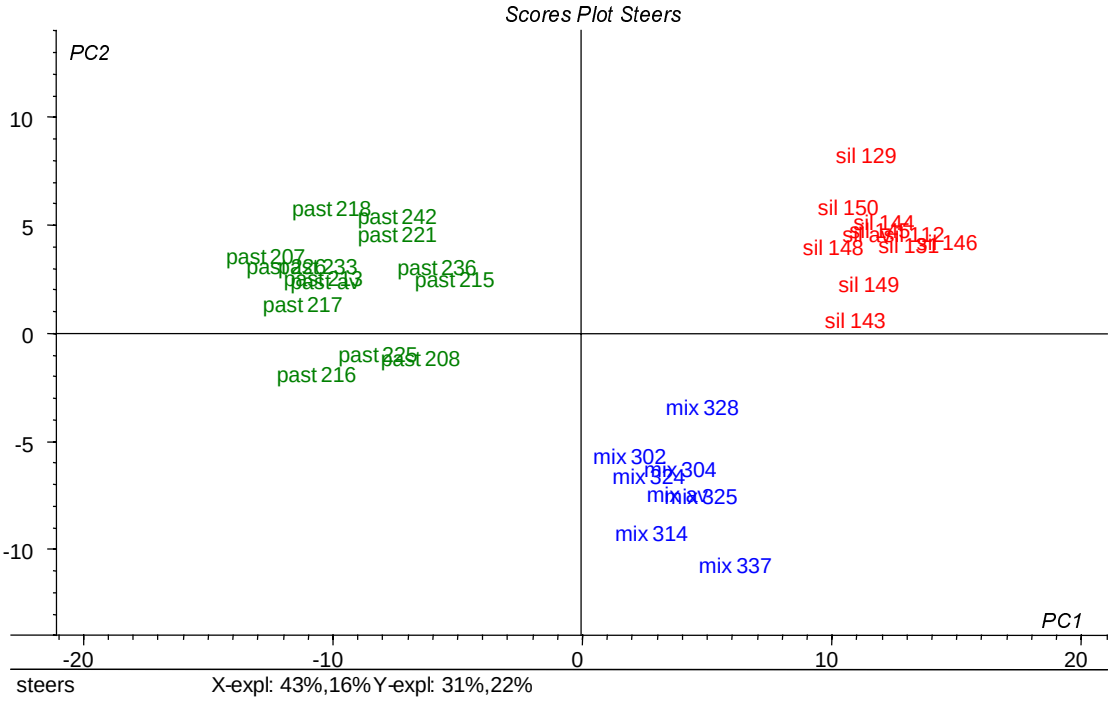


Figure A-3 : Steers – Scores-Plot of PLSR2 Data Analysis



8.8 Salami Experiments

8.8.1 Results of Tocopherol Determinations

Table A-20: Salami I - Mean and Standard Deviation of α-Tocopherol in Salami

normal ripening	control group 40		feeding group 100		feeding group 200	
	mean	deviation	mean	deviation	mean	deviation
week 2	$4.02^{a}{}_{A}$	0.08	$6.02^{b}{}_{A}$	0.17	$6.47^{c}{}_{A}$	0.18
week 4	$4.70^{a}{}_{B}$	0.11	n.d.	n.d.	$7.68^{b}{}_{B}$	0.08
week 11	$4.75^{a}{}_{B}$	0.11	$6.63^{b}{}_{B,C}$	0.50	$7.61^{c}{}_{B}$	0.20
week 16	$4.51^{a}{}_{B,C}$	0.15	$6.74^{b}{}_{B,C}$	0.30	$8.03^{c}{}_{B}$	0.51
week 21	$4.69^{a}{}_{B}$	0.13	$6.53^{b}{}_{B,C}$	0.17	$7.83^{c}{}_{B}$	0.25

special ripening	control group 40		feeding group 100		feeding group 200	
	mean	deviation	mean	deviation	mean	deviation
week 2	$4.09^{a}{}_{A}$	0.09	$5.99^{b}{}_{A}$	0.14	$6.80^{c}{}_{A}$	0.18
week 4	$4.49^{a}{}_{B}$	0.04	$6.82^{b}{}_{B}$	0.13	$7.78^{c}{}_{B}$	0.08
week 11	$4.54^{a}{}_{B,C}$	0.14	$6.97^{b}{}_{B}$	0.26	$7.84^{c}{}_{B,C}$	0.20
week 16	$4.74^{a}{}_{A,B,C}$	0.96	$7.07^{b}{}_{A,B}$	0.88	$8.29^{b}{}_{B,C}$	0.51
week 21	$4.70^{a}{}_{C}$	0.03	$6.96^{b}{}_{A,B}$	1.02	$8.13^{b}{}_{C}$	0.25

Mean values with different superscripts in a row are significantly ($p < 0.05$) different.
Mean values with different subscripts in a column are significantly different ($p < 0.05$).

Table A-21: Salami II - Mean and Standard Deviation of α-Tocopherol in Salami

	Control #1		Control #2		Control #3	
	mean	deviation	mean	deviation	mean	deviation
week 0	$4.05^{a}{}_{A}$	0.04	$3.85^{a}{}_{A}$	0.16	$4.83^{b}{}_{A}$	0.00
week 4	$3.43^{a}{}_{B}$	0.15	$3.66^{b}{}_{A}$	0.08	$3.72^{b}{}_{B}$	0.07
week 12	$3.41^{a}{}_{B}$	0.13	$3.56^{a}{}_{A}$	0.11	$4.22^{b}{}_{C}$	0.05
week 21	$3.09^{a}{}_{C}$	0.19	$3.27^{a}{}_{B}$	0.15	$3.22^{a}{}_{D}$	0.06

	Suppl. #1		Suppl. #2	
	mean	deviation	mean	deviation
week 0	$4.68^{b}{}_{C}$	0.14	$4.99^{b}{}_{A}$	0.14
week 4	$4.49^{c}{}_{A}$	0.26	$4.61^{c}{}_{B}$	0.04
week 12	$4.43^{b}{}_{A}$	0.23	$4.97^{c}{}_{A}$	0.05
week 21	$3.79^{b}{}_{B}$	0.12	$3.68^{b}{}_{C}$	0.03

Mean values with different superscripts in a row are significantly ($p < 0.05$) different.
Mean values with different subscripts in a column are significantly different ($p < 0.05$).

8.8.2 Results of Fatty Acid Determinations

Table A-22: Salami I – Mean Values of Total Fat I

Table A-23: Salami I – Mean Values of Total Fat II

Table A-24: Salami I – Mean Values of Phospholipids I

Table A-25: Salami I – Mean Values of Phospholipids II

Table A-26: Salami II – Mean Values of Total Fat and Phospholipids in Salamis with Rosemary

Table A-22: Salami I – Mean Values of Total Fat I

Fatty Acid	40 mg									
	week 0		week 2		week 11		week 16		week 21	
	NR	SR	NR	SR	NR	SR	NR	SR	NR	SR
C 10	0,09	0,09	0,09	0,08	0,08	0,09	0,06	0,06	0,09	0,06
C 12	0,08	0,10	0,12	0,10	0,10	0,11	0,09	0,09	0,11	0,09
C 14	1,50	1,47	1,57	1,49	1,49	1,53	1,28	1,28	1,49	1,33
C 15	0,07	0,08	0,07	0,06	0,07	0,06	0,06	0,05	0,07	0,06
C 16	22,62	23,04	23,06	23,23	23,29	23,67	22,08	22,48	23,54	22,80
C 16:1 c9	3,01	2,54	3,05	3,06	3,05	2,79	2,59	2,47	2,78	2,55
C 17	0,80	0,38	0,53	0,35	0,36	0,39	0,40	0,40	0,41	0,39
C 17:1	0,43	0,48	0,48	0,41	0,41	0,41	0,40	0,38	0,41	0,37
C 18	11,72	12,37	11,61	12,48	12,21	12,95	12,86	13,78	12,44	13,65
C 18:1 c9	39,03	39,61	39,08	38,98	39,23	39,33	40,53	40,08	39,68	40,05
C 18:2	10,32	9,64	9,95	9,48	9,79	9,39	9,96	9,42	9,62	9,33
C 18:3 c6	0,06	0,09	0,06	0,07	0,13	0,08	0,09	0,09	0,09	0,11
C 18:3 c9	1,01	0,92	1,02	1,02	0,93	0,88	0,94	0,89	0,91	0,87
C 20	0,20	0,20	0,22	0,21	0,22	0,19	0,20	0,20	0,17	0,20
C 20:1 c11	0,92	0,83	0,80	0,92	0,86	0,86	0,87	0,84	0,73	0,85
C 20:2 c11	0,64	0,51	0,60	0,72	0,72	0,48	0,46	0,46	0,41	0,45
C 20:3 c8	0,12	0,10	0,10	0,12	0,16	0,08	0,09	0,09	0,08	0,08
C 20:4	0,29	0,24	0,27	0,29	0,26	0,23	0,24	0,23	0,20	0,22
C 20:3 c11	0,19	0,16	0,17	0,16	0,16	0,16	0,17	0,17	0,16	0,16
C 22:4	0,08	0,07	0,08	0,07	0,07	0,08	0,08	0,08	0,08	0,08
C 22:5	0,15	0,12	0,14	0,14	0,12	0,12	0,14	0,14	0,12	0,12
C 22:6	0,06	0,05	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,05
∑ SFA	37,08	37,73	37,27	38,00	37,82	38,99	37,03	38,34	38,32	38,58
∑ MUFA	43,39	43,46	43,41	43,37	43,55	43,39	44,39	43,77	43,60	43,82
∑ PUFA	12,92	11,90	12,45	12,13	12,40	11,56	12,23	11,63	11,73	11,47

all values expressed as percentage of total fatty acids

Table A-23: Salami I – Mean Values of Total Fat II

Fatty Acid	100 mg									
	week 0		week 2		week 11		week 16		week 21	
	NR	SR	NR	SR	NR	SR	NR	SR	NR	SR
C 10	0,09	0,09	0,07	0,07	0,07	0,08	0,05	0,06	0,07	0,05
C 12	0,09	0,11	0,10	0,10	0,11	0,10	0,08	0,09	0,09	0,09
C 14	1,58	1,61	1,57	1,51	1,59	1,59	1,27	1,39	1,41	1,38
C 15	0,07	0,07	0,07	0,06	0,06	0,06	0,06	0,06	0,07	0,06
C 16	23,31	23,61	23,51	23,14	24,27	24,23	22,47	23,18	23,29	22,78
C 16:1 c9	3,07	2,74	2,86	3,32	3,29	2,94	2,58	2,58	2,73	2,58
C 17	0,40	0,37	0,53	0,38	0,39	0,37	0,39	0,39	0,39	0,30
C 17:1	0,42	0,46	0,48	0,42	0,39	0,41	0,41	0,40	0,40	0,39
C 18	11,99	12,15	12,06	12,30	12,42	12,66	13,35	13,48	13,19	13,45
C 18:1 c9	39,58	39,26	39,32	39,39	39,02	39,03	40,39	39,86	40,10	40,43
C 18:2	9,26	9,31	9,13	9,17	8,78	8,96	9,10	8,87	8,84	9,03
C 18:3 c6	0,07	0,06	0,07	0,06	0,17	0,07	0,09	0,09	0,08	0,08
C 18:3 c9	0,89	0,89	0,92	1,02	0,98	0,85	0,86	0,85	0,84	0,85
C 20	0,21	0,20	0,22	0,21	0,27	0,20	0,20	0,20	0,19	0,21
C 20:1 c11	0,93	0,90	0,90	0,91	0,89	0,87	0,87	0,82	0,81	0,86
C 20:2 c11	0,53	0,50	0,56	0,56	0,59	0,51	0,45	0,43	0,41	0,44
C 20:3 c8	0,11	0,10	0,10	0,11	0,16	0,12	0,10	0,09	0,10	0,09
C 20:4	0,27	0,24	0,26	0,26	0,23	0,24	0,23	0,23	0,22	0,22
C 20:3 c11	0,17	0,16	0,15	0,16	0,13	0,15	0,16	0,16	0,15	0,16
C 22:4	0,08	0,08	0,08	0,06	0,07	0,07	0,08	0,08	0,10	0,08
C 22:5	0,14	0,14	0,15	0,15	0,12	0,12	0,15	0,14	0,14	0,14
C 22:6	0,06	0,05	0,06	0,06	0,10	0,05	0,06	0,06	0,06	0,06
∑ SFA	37,74	38,21	38,13	37,77	39,18	39,29	37,87	38,85	38,70	38,32
∑ MUFA	44,00	43,36	43,56	44,04	43,59	43,25	44,25	43,66	44,04	44,26
∑ PUFA	11,58	11,53	11,48	11,61	11,33	11,14	11,28	11,00	10,94	11,15

Fatty Acid	200 mg									
	week 0		week 2		week 11		week 16		week 21	
	NR	SR	NR	SR	NR	SR	NR	SR	NR	SR
C 10	0,09	0,09	0,08	0,07	0,08	0,07	0,05	0,06	0,08	0,06
C 12	0,16	0,11	0,11	0,10	0,11	0,10	0,09	0,09	0,10	0,09
C 14	1,49	1,53	1,53	1,47	1,54	1,52	1,27	1,31	1,45	1,27
C 15	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,07	0,06
C 16	22,90	22,84	23,30	22,59	23,77	23,41	22,25	22,38	23,46	22,12
C 16:1 c9	3,00	2,94	2,88	3,27	3,03	3,04	2,58	2,63	2,84	2,66
C 17	0,33	0,32	0,46	0,32	0,33	0,33	0,35	0,35	0,36	0,35
C 17:1	0,39	0,44	0,41	0,38	0,36	0,36	0,36	0,36	0,38	0,37
C 18	12,02	11,50	12,07	11,74	12,46	12,01	13,22	12,93	12,95	12,85
C 18:1 c9	38,95	39,44	39,08	39,47	39,11	39,43	40,41	40,21	39,53	40,62
C 18:2	9,90	10,29	9,69	10,17	9,50	9,98	9,77	9,92	9,60	9,98
C 18:3 c6	0,07	0,06	0,06	0,06	0,15	0,07	0,09	0,09	0,08	0,09
C 18:3 c9	0,97	0,98	0,93	1,18	0,91	0,95	0,30	0,94	0,91	0,94
C 20	0,20	0,20	0,22	0,21	0,22	0,24	0,19	0,20	0,18	0,20
C 20:1 c11	0,89	0,88	0,87	0,90	0,87	0,89	0,81	0,83	0,76	0,85
C 20:2 c11	0,62	0,52	0,53	0,61	0,62	0,58	0,45	0,47	0,43	0,48
C 20:3 c8	0,11	0,11	0,11	0,11	0,17	0,14	0,10	0,10	0,09	0,09
C 20:4	0,26	0,28	0,27	0,29	0,24	0,23	0,23	0,24	0,21	0,22
C 20:3 c11	0,17	0,17	0,17	0,17	0,15	0,16	0,17	0,19	0,16	0,17
C 22:4	0,08	0,10	0,08	0,08	0,07	0,08	0,08	0,08	0,08	0,08
C 22:5	0,14	0,18	0,14	0,15	0,12	0,12	0,14	0,15	0,12	0,15
C 22:6	0,05	0,05	0,05	0,06	0,06	0,05	0,06	0,06	0,05	0,06
∑ SFA	37,25	36,66	37,83	36,56	38,57	37,74	37,48	37,38	38,65	37,00
∑ MUFA	43,23	43,70	43,24	44,02	43,37	43,72	44,16	44,03	43,51	44,50
∑ PUFA	12,37	12,74	12,03	12,88	11,99	12,36	11,39	12,24	11,73	12,26

all values expressed as percentage of total fatty acids

Table A-24: Salami I – Mean Values of Phospholipids I

Fatty Acid	40 mg									
	week 0		week 2		week 11		week 16		week 21	
	NR	SR	NR	SR	NR	SR	NR	SR	NR	SR
C 14	0,15	0,46	0,48	0,55	0,22	0,27	0,49	0,20	0,26	0,24
C 15	0,09	0,12	0,14	0,11	0,10	0,12	0,06	0,07	0,06	0,08
C 16	16,42	19,27	18,49	18,80	14,77	17,55	13,44	9,88	10,58	11,63
C 16:1 c9	1,37	1,54	1,39	1,69	1,17	1,42	1,49	1,00	1,05	1,12
C 17	0,35	0,40	0,38	0,38	0,38	0,46	0,37	0,32	0,33	0,35
C 17:1	0,32	1,28	1,22	0,96	0,64	0,76	0,51	0,51	0,61	0,75
C 18	15,85	14,98	15,59	16,09	18,70	16,84	18,12	18,65	19,90	20,09
C 18:1 c9	18,20	18,27	18,45	19,27	19,76	20,16	24,19	18,58	19,81	19,76
C 18:2	25,59	24,03	22,40	20,68	20,54	19,26	19,25	24,77	22,08	21,51
C 18:3 c6	0,25	0,30	0,23	0,29	0,23	0,20	0,24	0,31	0,25	0,23
C 18:3 c9	0,78	0,77	0,67	0,68	0,59	0,72	0,63	0,73	0,65	0,60
C 20	0,11	0,11	0,13	0,36	0,10	0,68	0,14	0,14	0,18	0,13
C 20:1 c11	0,23	0,16	0,32	0,26	0,20	0,44	0,35	0,28	0,38	0,30
C 20:2 c11	0,51	0,36	0,50	0,65	0,47	0,99	0,52	0,60	0,61	0,56
C 20:3 c5	0,57	0,51	0,70	0,62	0,87	0,68	0,77	0,98	0,98	0,99
C 20:3 c8	1,25	0,99	1,20	0,99	1,31	1,07	1,19	1,49	1,47	1,38
C 20:4	8,12	6,93	7,67	7,11	9,54	0,90	8,00	10,74	9,98	9,85
C 20:3 c11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,18	0,20	0,17	0,16
C 20:5	0,47	0,46	0,42	0,39	0,45	0,44	0,42	0,60	0,52	0,48
C 22	n.d.	n.d.	n.d.	n.d.	n.d.	0,10	0,07	0,08	0,10	0,08
C 22:4	1,03	0,69	0,86	0,71	1,12	0,99	1,14	1,51	1,45	1,21
C 22:5	1,35	0,87	1,00	0,81	1,21	1,08	1,17	1,59	1,53	1,24
C 22:6	0,58	0,40	0,44	0,38	0,49	0,43	0,40	0,50	0,50	0,45
∑ SFA	32,97	35,34	35,21	36,29	34,27	36,02	32,69	29,34	31,41	32,60
∑ MUFA	20,12	21,25	21,38	22,18	21,77	22,78	26,54	20,37	21,85	21,93
∑ PUFA	39,25	35,32	34,89	32,32	35,51	25,69	32,72	42,53	38,72	37,28

Fatty Acid	100 mg									
	week 0		week 2		week 11		week 16		week 21	
	NR	SR	NR	SR	NR	SR	NR	SR	NR	SR
C 14	0,42	0,44	0,48	0,60	0,22	0,25	0,23	0,20	0,38	0,22
C 15	0,08	0,10	0,10	0,10	0,10	0,11	0,06	0,07	0,06	0,07
C 16	17,78	18,94	19,02	20,07	15,73	17,30	11,53	10,66	12,20	11,46
C 16:1 c9	1,61	1,41	1,43	1,62	1,19	1,43	1,02	0,97	1,28	1,01
C 17	0,33	0,37	0,35	0,31	0,37	0,34	0,34	0,31	0,30	0,33
C 17:1	0,54	1,11	1,19	1,18	0,57	1,26	0,55	0,50	0,51	0,70
C 18	14,24	14,74	14,98	15,58	18,51	16,30	19,32	19,09	18,14	19,83
C 18:1 c9	18,74	18,05	18,06	18,62	19,19	19,99	19,38	18,93	21,69	19,07
C 18:2	24,79	24,80	22,55	20,42	21,91	20,48	22,98	24,17	21,56	23,75
C 18:3 c6	0,25	0,26	0,33	0,25	0,24	0,21	0,29	0,29	0,24	0,24
C 18:3 c9	0,81	0,80	0,76	0,68	0,61	0,67	0,65	0,72	0,67	0,69
C 20	0,16	0,09	0,20	0,33	0,10	0,52	0,13	0,15	0,14	0,14
C 20:1 c11	0,33	0,16	0,40	0,36	0,21	0,42	0,31	0,35	0,40	0,30
C 20:2 c11	0,58	0,40	0,56	0,76	0,48	0,98	0,60	0,63	0,59	0,60
C 20:3 c5	0,56	0,56	0,71	0,64	0,86	0,73	0,93	0,98	1,11	0,99
C 20:3 c8	1,18	1,13	1,18	1,04	1,29	1,18	1,44	1,55	1,15	1,47
C 20:4	7,40	7,00	7,23	6,69	8,70	7,63	9,27	9,77	8,82	9,25
C 20:3 c11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,18	0,20	0,16	0,16
C 20:5	0,48	0,45	0,46	0,36	0,43	0,40	0,50	0,53	0,70	0,47
C 22	n.d.	n.d.	0,09	n.d.	n.d.	n.d.	0,08	0,08	0,08	0,09
C 22:4	0,85	0,78	0,81	0,67	1,05	0,97	1,38	1,47	1,29	1,33
C 22:5	1,12	0,98	0,92	0,78	1,09	1,07	1,43	1,52	1,30	1,34
C 22:6	0,49	0,41	0,55	0,34	0,39	0,43	0,40	0,43	0,40	0,40
∑ SFA	33,01	34,68	35,22	36,99	35,03	34,82	31,69	30,56	31,30	32,14
∑ MUFA	21,22	20,73	21,08	21,78	21,16	23,10	21,26	20,75	23,88	21,08
∑ PUFA	37,33	36,44	34,88	31,59	35,76	33,57	38,61	40,71	36,84	39,22

all values expressed as percentage of total fatty acids

Table A-25: Salami I – Mean Values of Phospholipids II

Fatty Acid	200 mg									
	week 0		week 2		week 11		week 16		week 21	
	NR	SR	NR	SR	NR	SR	NR	SR	NR	SR
C 14	0,35	0,43	0,55	0,53	0,28	0,24	0,40	0,21	0,35	0,30
C 15	0,09	0,11	0,09	0,09	0,08	0,08	0,06	0,06	0,05	0,12
C 16	17,31	19,41	18,46	18,29	14,35	14,27	12,65	9,87	11,26	11,73
C 16:1 c9	1,53	1,36	1,58	1,45	1,17	1,49	1,27	0,98	1,17	1,12
C 17	0,30	0,36	0,32	0,33	0,33	0,28	0,32	0,26	0,29	0,31
C 17:1	0,48	1,35	1,14	0,97	0,56	1,14	0,68	0,56	0,64	0,62
C 18	14,34	14,80	15,07	15,57	18,52	17,95	17,94	17,88	18,40	19,60
C 18:1 c9	16,77	17,63	17,83	18,00	18,76	18,69	21,46	18,54	20,21	19,46
C 18:2	26,54	24,80	23,22	22,47	23,44	21,66	22,04	25,84	23,06	23,02
C 18:3 c6	0,25	0,33	0,29	0,24	0,21	0,14	0,26	0,29	0,22	0,21
C 18:3 c9	0,87	0,77	0,70	0,67	0,63	0,73	0,64	0,75	0,75	0,65
C 20	0,15	0,15	0,16	0,40	0,11	0,82	0,13	0,14	0,14	0,14
C 20:1 c11	0,24	0,15	0,30	0,29	0,20	0,49	0,33	0,29	0,32	0,32
C 20:2 c11	0,61	0,37	0,48	0,70	0,51	1,11	0,55	0,63	0,59	0,61
C 20:3 c5	0,56	0,51	0,65	0,62	0,82	0,89	0,80	0,92	0,86	0,97
C 20:3 c8	1,22	1,09	1,14	1,09	1,31	1,29	1,29	1,55	1,38	1,37
C 20:4	8,00	6,77	7,37	7,19	9,09	8,60	8,79	10,45	9,41	9,56
C 20:3 c11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,18	0,22	0,18	0,17
C 20:5	0,50	0,38	0,41	0,37	0,41	0,38	0,45	0,56	0,48	0,45
C 22	n.d.	n.d.	0,09	n.d.	n.d.	n.d.	0,09	0,07	0,07	0,09
C 22:4	0,90	0,70	0,78	0,79	1,08	1,05	1,26	1,55	1,33	1,16
C 22:5	1,25	0,88	0,98	0,92	1,18	1,09	1,37	1,66	1,44	1,22
C 22:6	0,51	0,39	0,43	0,39	0,44	0,40	0,41	0,50	0,43	0,44
∑ SFA	32,54	35,26	34,74	35,21	33,67	33,64	31,59	28,49	30,56	32,29
∑ MUFA	19,02	20,49	20,85	20,71	20,69	21,81	23,74	20,37	22,34	21,52
∑ PUFA	39,99	35,90	35,31	34,36	37,81	36,05	36,75	43,37	38,75	38,46

all values expressed as percentage of total fatty acids

Table A-26: Salami II - Mean Values of Total Fat and Phospholipids in Salamis with Rosemary

Fatty Acid	total fat									
	Control #1		Control #2		Control #3		Suppl. #1		Suppl. #2	
	week 0	week 12	week 0	week 12	week 0	week 12	week 0	week 12	week 0	week 12
C 10	0,09	0,00	0,10	0,09	0,09	0,00	0,10	0,00	0,10	0,10
C 12	0,10	0,00	0,09	0,10	0,09	0,00	0,10	0,00	0,10	0,07
C 14	1,53	1,46	1,55	1,48	1,51	1,48	1,57	1,53	1,65	1,59
C 15	0,06	0,00	0,10	0,09	0,07	0,00	0,00	0,00	0,00	0,00
C 16	24,05	22,97	24,18	22,93	24,14	23,12	24,23	23,52	24,60	23,55
C 16:1 t	0,34	0,33	0,30	0,35	0,32	0,26	0,28	0,31	0,28	0,28
C 16:1	2,40	2,30	2,40	2,35	2,39	2,08	2,78	2,70	2,93	2,82
C 17	0,39	0,34	0,39	0,34	0,38	0,35	0,28	0,24	0,28	0,21
C 17:1	0,33	0,27	0,33	0,29	0,31	0,26	0,26	0,22	0,26	0,20
C 18	11,88	12,53	11,80	12,07	12,04	12,88	11,09	11,17	10,91	11,63
C 18:1 t	0,07	0,00	0,04	0,49	0,00	0,00	0,00	0,00	0,06	0,00
C 18:1 c9	33,81	34,81	33,94	34,98	32,78	34,63	36,67	37,27	35,66	36,29
C 18:1 c11	4,35	6,06	4,12	5,54	5,21	6,26	4,26	6,50	4,86	6,94
C 18:2	16,55	15,54	16,42	15,28	16,48	15,07	14,64	13,85	14,56	13,20
C 18:3 c6	0,12	0,00	0,10	0,00	0,12	0,00	0,00	0,00	0,10	0,00
C 18:3 c9	1,60	1,47	1,57	1,46	1,59	1,42	1,42	1,31	1,40	1,27
C 20	0,10	0,00	0,24	0,23	0,15	0,21	0,09	0,00	0,09	0,00
C 20:1 c11	0,60	0,69	0,60	0,67	0,56	0,70	0,59	0,48	0,60	0,76
C 20:2	0,62	0,66	0,71	0,71	0,72	0,73	0,65	0,62	0,54	0,58
C 20:3 c8	0,11	0,00	0,10	0,00	0,11	0,00	0,10	0,00	0,10	0,00
C 20:4	0,37	0,33	0,38	0,34	0,38	0,33	0,38	0,26	0,42	0,30
C 20:3 c11	0,28	0,24	0,27	0,23	0,29	0,23	0,26	0,00	0,25	0,21
C 22:4	0,11	0,00	0,12	0,00	0,12	0,00	0,11	0,00	0,11	0,00
C 22:5	0,14	0,00	0,14	0,00	0,15	0,00	0,14	0,00	0,14	0,00
∑ SFA	38,20	37,30	38,44	37,32	38,49	38,03	37,45	36,46	37,73	37,16
∑ MUFA	41,90	44,46	41,74	44,66	41,57	44,19	44,85	47,48	44,65	47,28
∑ PUFA	3,35	2,70	3,39	2,74	3,47	2,71	3,05	2,20	3,06	2,36

Fatty Acid	phospholipids									
	Control #1		Control #2		Control #3		Suppl. #1		Suppl. #2	
	week 0	week 12	week 0	week 12	week 0	week 12	week 0	week 12	week 0	week 12
C 12	0,00	0,10	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,08
C 14	0,16	0,27	0,16	0,18	0,16	0,25	0,18	0,27	0,22	0,19
C 15	0,10	0,11	0,09	0,08	0,08	0,11	0,09	0,06	0,08	0,15
C 16	19,17	14,97	18,94	13,58	18,75	14,25	19,35	14,56	19,30	13,40
C 16:1 t	0,10	0,13	0,11	0,11	0,10	0,15	0,10	0,13	0,11	0,11
C 16:1	0,46	0,49	0,45	0,38	0,46	0,51	0,48	0,49	0,56	0,40
C 17	0,30	0,31	0,32	0,27	0,30	0,28	0,29	0,25	0,30	0,26
C 17:1	0,08	0,05	0,09	0,07	0,09	0,00	0,09	0,06	0,11	0,06
C 18	16,93	18,18	17,16	19,67	17,00	18,79	16,84	18,85	16,86	19,85
C 18:1 t	0,04	0,00	0,12	0,10	0,10	0,00	0,11	0,00	0,12	0,00
C 18:1 c9	8,75	10,25	8,63	9,05	8,75	10,07	8,91	10,14	9,59	9,50
C 18:1 c11	3,92	4,42	3,89	4,10	3,98	4,22	3,96	4,47	3,91	4,38
C 18:2	31,44	26,68	31,19	26,78	31,25	26,52	31,15	26,38	31,14	26,67
C 18:3 c6	0,21	0,28	0,23	0,20	0,22	0,30	0,22	0,28	0,24	0,32
C 18:3 c9	0,69	0,58	0,71	0,54	0,71	0,55	0,71	0,56	0,77	0,54
C 20	0,05	0,13	0,05	0,13	0,05	0,13	0,05	0,13	0,05	0,13
C 20:1 c11	0,25	0,30	0,25	0,33	0,26	0,32	0,26	0,44	0,27	0,33
C 20:2	0,65	0,75	0,64	0,98	0,62	0,72	0,61	0,74	0,61	0,79
C 20:3 c8	1,07	1,22	1,05	1,47	1,06	1,20	1,05	1,33	1,03	1,38
C 20:4	12,97	17,17	13,15	18,21	13,32	17,78	12,93	17,21	12,14	17,46
C 20:5	0,56	0,40	0,55	0,42	0,56	0,42	0,53	0,38	0,56	0,43
C 22:4	0,68	1,25	0,72	1,21	0,70	1,19	0,60	1,25	0,58	1,30
C 22:5	1,43	1,98	1,47	2,08	1,47	2,10	1,45	1,98	1,46	2,20
C 22:6	0,59	0,00	0,58	0,00	0,57	0,00	0,54	0,64	0,58	0,73
∑ SFA	36,71	34,07	36,72	33,91	36,33	33,89	36,79	34,11	36,82	34,06
∑ MUFA	13,60	15,64	13,52	14,14	13,73	15,28	13,91	15,73	14,66	14,78
∑ PUFA	50,28	50,30	50,29	51,89	50,47	50,78	49,80	50,75	49,10	51,83

all values expressed as percentage of total fatty acids

CURRICULUM VITAE MAIKE TIMM

Personal Data

Date, place of birth:	24th of September 1968, Hamburg, Germany

School

Aug 1974 - Jun 1978	Primary school: Grundschule Scheesseler Kehre, Hamburg
Aug 1978 - May 1987	Secondary school: Gymnasium Sinstorf, Hamburg, Graduation: A-Level (Abitur - university entrance qualification)

University

Oct 1987 - Feb 1993	Studies of Food Chemistry, University of Hamburg
Mar 1993 - Apr 1994	Diploma "Trans-Fatty Acids in Foods", Graduation First State Examination
May 1994 - Jul 1994	Second State Examination / Graduation as "State-Certified Food Chemist"
Nov 1994 - Dec 1999	PhD student at the Department of Food Chemistry, University of Hamburg

Professional Experience

Mar 1988	SGS Control Co KG - Lab. for Food and Environmental Analysis, Hamburg
May 1993 - Oct 1993	Dr. Kaiser & Dr. Woldmann – Lab. for Food and Environmental Analysis, Hamburg
Nov 1993 - Apr 1994	State's Food Control Laboratory, Hamburg
Nov 1994 - Dec 1997	University of Hamburg, lectures and beginners course in gas chromatography
Since Jan 2000	Research Assistant, Danish Institute for Fisheries Research, Copenhagen / Denmark

Further Training

Dec 1998 – Nov 1999	"Scandinavia Management" (business administration, management), Lübeck

International Job Placements

Aug 1990 - Sep 1990	Chemical Research Institute, Ljubljana / Slovenia Synthesis of compounds with potential anticancerogenic effect
Aug 1991 - Oct 1991	University of Campinas, Fac. of Food Engineering, Campinas / Brazil Production of xanthan gum for the application in cheese making
Aug 1994 - Oct 1994	CDCC - Univ. of Sao Paulo, Dept. of Chemistry, Sao Carlos / Brazil Development of chemical experiments for children to perform at home
Jun 1997	University College Cork, Dept. of Food Technology, Cork / Ireland Influence of vitamin E on meat quality
May 1998 - Sept 1998	MD Foods (Dairy Company), R & D Centre, Århus / Denmark Fat globule damage - new methods for raw milk samples
Sept 1999 - Nov 1999	Danish Institute for Fisheries Research, Copenhagen / Denmark Development of a CE-method to determine volatile amines in fish

Voluntary Work

Oct 1994 – Aug 1999	Work for IAESTE (International Association for the Exchange of Students for Technical Experience), in Hamburg, nation-wide and international

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Danish	Advanced level
Portuguese	Advanced level
Swedish	Basic knowledge